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(71) Applicants: CREATIVE BIOMOLECULES, INC. [US/US]; 35 South Street, Hopkinton, MA 01748 (US). CETUS ONCOLOGY CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608-2916 (US).

(72) Inventors: HUSTON, James, S. ; Five Drew Road, Chestnut Hill, MA 02167 (US). HOUSTON, L., L. ; 1901 Oakcrest Drive, Oakland, CA 94602 (US). RING, David, B. ; 1248 Truman Street, Redwood City, CA 94061 (US). OPPERMANN, Hermann ; 23 Summer Hill Road, Medway, MA 02053 (US).

(74) Agent: KELLEY, Robin, D.; Testa, Hurwitz & Thibeault, Exchange Place, 53 State Street, Boston, MA 02109 (US).

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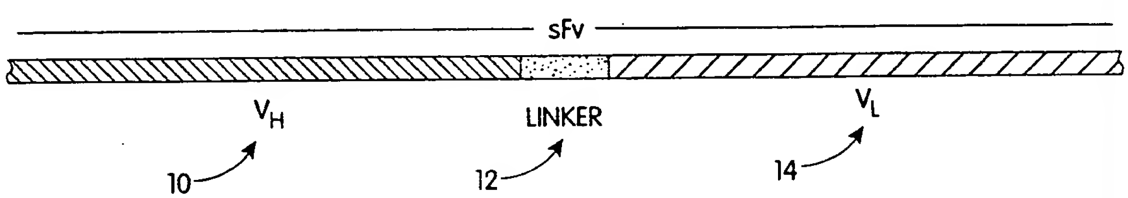

(57) Abstract

Disclosed is a single-chain Fv (sFv) polypeptide defining a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen, the sFv includes at least two polypeptide domains connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each of the polypeptide domains includes a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), the CDRs conferring immunological binding to the c-erbB-2 or c-erbB-2-related tumor antigen.

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BIOSYNTHETIC BINDING PROTEIN FOR CANCER MARKER

This invention relates in general to novel biosynthetic compositions of matter and, specifically, to biosynthetic antibody binding site (BABS) proteins, and conjugates thereof. Compositions of the invention are useful, for example, in drug and toxin targeting, imaging, immunological treatment of various cancers, and in specific binding assays, affinity purification schemes, and biocatalysis.

Background of the Invention

Carcinoma of the breast is the most common malignancy among women in North America, with 130,000 new cases in 1987. Approximately one in 11 women develop breast cancer in their lifetimes, causing this malignancy to be the second leading cause of cancer death among women in the United States, after lung cancer. Although the majority of women with breast cancer present with completely resectable disease, metastatic disease remains a formidable obstacle to cure. The use of adjuvant chemotherapy or hormonal therapy has definite positive impact on disease-free survival and overall survival in selected subsets of women with completely resected primary breast cancer, but a substantial proportion of women still relapse with metastatic disease (see, e.g., Fisher et al. (1986) J. Clin. Oncol. 4:929-941; "The Scottish trial", Lancet (1987) 2:171-175). In spite of the regularly induced objective responses induced by chemotherapy and hormonal therapy in appropriately selected patients, cure of metastatic breast cancer has not been achieved (see e.g., Aisner, et al. (187) J. Clin. Oncol.

5:1523-1533). To this end, many innovative treatment programs including the use of new agents, combinations of agents, high dose therapy (Henderson, ibid.) and increased dose intensity (Kernan et al. (1988) Clin. Invest. 259:3154-3157) have been assembled. Although improvements have been observed, routine achievement of complete remissions of metastatic disease, the first step toward cure, has not occurred. There remains a pressing need for new approaches to treatment.

The Fv fragment of an immunoglobulin molecule from IgM, and on rare occasions IgG or IgA, is produced by proteolytic cleavage and includes a non-covalent V_H-V_L heterodimer representing an intact antigen binding site. A single chain Fv (sFv) polypeptide is a covalently linked V_H-V_L heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes connected by a peptide-encoding linker. See Huston et al., 1988, Proc. Nat. Aca. Sci. 85: 5879, hereby incorporated by reference.

U.S. Patent 4,753,894 discloses murine monoclonal antibodies which bind selectively to human breast cancer cells and, when conjugated to ricin A chain, exhibit a TCID 50% against at least one of MCF-7, CAMA-1, SKBR-3, or BT-20 cells of less than about 10 nM. The SKBR-3 cell line is recognized specifically by the monoclonal antibody 520C9. The antibody designated 520C9 is secreted by a murine hybridoma and is now known to recognize c-erbB-2 (Ring et al., 1991, Molecular Immunology 28:915).

Summary of the Invention

The invention features the synthesis of a class of novel proteins known as single chain Fv (sFv) polypeptides, which include biosynthetic single polypeptide chain binding sites (BABS) and define a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen.

The sFv includes at least two polypeptide domains connected by a polypeptide linker spanning the distance between the carboxy (C)- terminus of one domain and the amino (N)- terminus of the other domain, the amino acid sequence of each of the polypeptide domains including a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), the CDRs conferring immunological binding to c-erbB-2 or a c-erbB-2 related tumor antigen.

In its broadest aspects, this invention features single-chain Fv polypeptides including biosynthetic antibody binding sites, replicable expression vectors prepared by recombinant DNA techniques which include and are capable of expressing DNA sequences encoding these polypeptides, methods for the production of these polypeptides, methods of imaging a tumor expressing c-erbB-2 or a c-erbB-2-related tumor antigen, and methods of treating a tumor using targetable therapeutic agents by virtue of conjugates or fusions with these polypeptides.

As used herein, the term "immunological binding" or "immunologically reactive" refers to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific; "c-erbB-2" refers to a

protein antigen expressed on the surface of tumor cells, such as breast and ovarian tumor cells, which is an approximately 200,000 molecular weight acidic glycoprotein having an isoelectric point of about 5.3 and including the amino acid sequence set forth in SEQ ID NOS:1 and 2. A "c-erbB-2-related tumor antigen" is a protein located on the surface of tumor cells, such as breast and ovarian tumor cells, which is antigenically related to the c-erbB-2 antigen, i.e., bound by an immunoglobulin that is capable of binding the c-erbB-2 antigen, examples of such immunoglobulins being the 520C9, 741F8, and 454C11 antibodies; or which has an amino acid sequence that is at least 80% homologous, preferably 90% homologous, with the amino acid sequence of c-erbB-2. An example of a c-erbB-2 related antigen is the receptor for epidermal growth factor.

An sFv CDR that is "substantially homologous with" an immunoglobulin CDR retains at least 70%, preferably 80% or 90%, of the amino acid sequence of the immunoglobulin CDR, and also retains the immunological binding properties of the immunoglobulin.

The term "domain" refers to that sequence of a polypeptide that folds into a single globular region in its native conformation, and may exhibit discrete binding or functional properties. The term "CDR" or complementarity determining region, as used herein, refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site, or a synthetic polypeptide which mimics this function. CDRs typically are not wholly homologous to hypervariable regions of natural Fvs, but rather may also include specific amino acids or amino acid sequences which

flank the hypervariable region and have heretofore been considered framework not directly determinative of complementarity. The term "FR" or framework region, as used herein, refers to amino acid sequences which are naturally found between CDRs in immunoglobulins.

Single-chain Fv polypeptides produced in accordance with the invention include biosynthetically-produced novel sequences of amino acids defining polypeptides designed to bind with a preselected c-erbB-2 or related antigen material. The structure of these synthetic polypeptides is unlike that of naturally occurring antibodies, fragments thereof, or known synthetic polypeptides or "chimeric antibodies" in that the regions of the single-chain Fv responsible for specificity and affinity of binding (analogous to native antibody variable (V_H/V_L) regions) may themselves be chimeric, e.g., include amino acid sequences derived from or homologous with portions of at least two different antibody molecules from the same or different species. These analogous V_H and V_L regions are connected from the N-terminus of one to the C-terminus of the other by a peptide bonded biosynthetic linker peptide.

The invention thus provides a single-chain Fv polypeptide defining at least one complete binding site capable of binding c-erbB-2 or a c-erbB-2-related tumor antigen. One complete binding site includes a single contiguous chain of amino acids having two polypeptide domains, e.g., V_H and V_L, connected by a amino acid linker region. An sFv that includes more than one complete binding site capable of binding a c-erbB-2-related antigen, e.g., two binding sites, will be a single contiguous chain of amino acids having four polypeptide domains, each of which is covalently linked

by an amino acid linker region, e.g., V_{H1}-linker-V_{L1}-linker-V_{H2}-linkerV_{L2}. sFv's of the invention may include any number of complete binding sites (V_{Hn}-linker-V_{Ln})$_n$, where n > 1, and thus may be a single contiguous chain of amino acids having n antigen binding sites and n X 2 polypeptide domains.

In one preferred embodiment of the invention, the single-chain Fv polypeptide includes CDRs that are substantially homologous with at least a portion of the amino acid sequence of CDRs from a variable region of an immunoglobulin molecule from a first species, and includes FRs that are substantially homologous with at least a portion of the amino acid sequence of FRs from a variable region of an immunoglobulin molecule from a second species. Preferably, the first species is mouse and the second species is human.

The amino acid sequence of each of the polypeptide domains includes a set of CDRs interposed between a set of FRs. As used herein, a "set of CDRs" refers to 3 CDRs in each domain, and a "set of FRS" refers to 4 FRs in each domain. Because of structural considerations, an entire set of CDRs from an immunoglobulin may be used, but substitutions of particular residues may be desirable to improve biological activity, e.g., based on observations of conserved residues within the CDRs of immunoglobulin species which bind c-erbB-2 related antigens.

In another preferred aspect of the invention, the CDRs of the polypeptide chain have an amino acid sequence substantially homologous with the CDRs of the variable region of any one of the 520C9, 741F8, and 454C11 monoclonal antibodies. The CDRs of the 520C9 antibody are set forth in the Sequence Listing as amino acid residue numbers 31 through 35, 50 through 66, 99

through 104, 159 through 169, 185 through 191, and 224 through 232 in SEQ ID NOS: 3 and 4, and amino acid residue numbers 31 through 35, 50 through 66, 99 through 104, 157 through 167, 183 through 189, and 222 through 230 in SEQ ID NOS: 5, and 6.

In one embodiment, the sFv is a humanized hybrid molecule which includes CDRs from the mouse 520C9 antibody interposed between FRs derived from one or more human immunoglobulin molecules. This hybrid sFv thus contains binding regions which are highly specific for the c-erbB-2 antigen or c-erbB-2-related antigens held in proper immunochemical binding conformation by human FR amino acid sequences, and thus will be less likely to be recognized as foreign by the human body.

In another embodiment, the polypeptide linker region includes the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 123 through 137 in SEQ ID NOS:3 and 4, and as amino acid residues 1-16 in SEQ ID NOS:11 and 12. In other embodiments, the linker sequence has the amino acid sequence set forth in the Sequence Listing as amino acid residues 121-135 in SEQ ID NOS:5 and 6, or the amino acid sequence of residues 1-15 in SEQ ID NOS:13 and 14.

The single polypeptide chain described above also may include a remotely detectable moiety bound thereto to permit imaging or radioimmunotherapy of tumors bearing a c-erbB-2 or related tumor antigen. "Remotely detectable" moiety means that the moiety that is bound to the sFv may be detected by means external to and at a distance from the site of the moiety. Preferable remotely detectable moieties for imaging include radioactive atom such as ^{99m}Technetium (^{99m}Tc), a gamma emitter. Preferable nucleotides for high dose

radioimmunotherapy include radioactive atoms such as, (^{90}Yttrium (^{90}Yt), ^{131}Iodine (^{131}I) or ^{111}Indium (^{111}In).

In addition, the sFv may include a fusion protein derived from a gene fusion, such that the expressed sFv fusion protein includes an ancillary polypeptide that is peptide bonded to the binding site polypeptide. In some preferred aspects, the ancillary polypeptide segment also has a binding affinity for a c-erbB-2 or related antigen and may include a third and even a fourth polypeptide domain, each comprising an amino acid sequence defining CDRs interposed between FRs, and which together form a second single polypeptide chain biosynthetic binding site similar to the first described above.

In other aspects, the ancillary polypeptide sequence forms a toxin linked to the N or C terminus of the sFv, e.g., at least a toxic portion of Pseudomonas exotoxin, phytolaccin, ricin, ricin A chain, or diphtheria toxin, or other related proteins known as ricin A chain-like ribosomal inhibiting proteins, i.e., proteins capable of inhibiting protein synthesis at the level of the ribosome, such as pokeweed antiviral protein, gelonin, and barley ribosomal protein inhibitor. In still another aspect, the sFv may include at least a second ancillary polypeptide or moiety which will promote internalization of the sFv.

The invention also includes a method for producing sFv, which includes the steps of providing a replicable expression vector which includes and which expresses a DNA sequence encoding the single polypeptide chain; transfecting the expression vector into a host cell to produce a transformant; and culturing the transformant to produce the sFv polypeptide.

The invention also includes a method of imaging a tumor expressing a c-erbB-2 or related tumor antigen. This method includes the steps of providing an imaging agent including a single-chain Fv polypeptide as described above, and a remotely detectable moiety linked thereto; administering the imaging agent to an organism harboring the tumor in an amount of the imaging agent with a physiologically-compatible carrier sufficient to permit extracorporeal detection of the tumor; and detecting the location of the moiety in the subject after allowing the agent to bind to the tumor and unbound agent to have cleared sufficiently to permit visualization of the tumor image.

The invention also includes a method of treating cancer by inhibiting in vivo growth of a tumor expressing a c-erbB-2 or related antigen, the method including administering to a cancer patient a tumor inhibiting amount of a therapeutic agent which includes an sFv of the invention and at least a first moiety peptide bonded thereto, and which has the ability to limit the proliferation of a tumor cell.

Preferably, the first moiety includes a toxin or a toxic fragment thereof, e.g., ricin A; or includes a radioisotope sufficiently radioactive to inhibit proliferation of the tumor cell, e.g., ^{90}Yt, ^{111}In, or ^{131}I. The therapeutic agent may further include at least a second moiety that improves its effectiveness.

The clinical administration of the single-chain Fv or appropriate sFv fusion proteins of the invention, which display the activity of native, relatively small Fv of the corresponding immunoglobulin, affords a number of advantages over the use of larger fragments or entire antibody molecules. The single chain Fv and sFv fusion proteins of this invention offer fewer

cleavage sites to circulating proteolytic enzymes and thus offer greater stability. They reach their target tissue more rapidly, and are cleared more quickly from the body, which makes them ideal imaging agents for tumor detection and ideal radioimmunotherapeutic agents for tumor killing. They also have reduced non-specific binding and immunogenicity relative to murine immunoglobulins. In addition, their expression from single genes facilitates targeting applications by fusion to other toxin proteins or peptide sequences that allow specific coupling to other molecules or drugs. In addition, some sFv analogues or fusion proteins of the invention have the ability to promote the internalization of c-erbB-2 or related antigens expressed on the surface of tumor cells when they are bound together at the cell surface. These methods permit the selective killing of cells expressing such antigens with the single-chain-Fv-toxin fusion of appropriate design. sFv-toxin fusion proteins of the invention possess 15-200-fold greater tumor cell killing activity than conjugates which include a toxin that is chemically crosslinked to whole antibody or Fab.

Overexpression of c-erbB-2 or related receptors on malignant cells thus allows targeting of sFv species to the tumor cells, whether the tumor is well-localized or metastatic. In the above cases, the internalization of sFv-toxin fusion proteins permits specific destruction of tumor cells bearing the over expressed c-erbB-2 or related antigen. In other cases, depending on the infected cells, the nature of the malignancy, or other factors operating in a given individual, the same c-erbB-2 or related receptors may be poorly internalized or even represent a static tumor antigen

population. In this event, the single-chain Fv and its fusion proteins can also be used productively, but in a different mode than applicable to internalization of the toxin fusion. Where c-erbB-2 receptor/sFv or sFv fusion protein complexes are poorly internalized, toxins, such as ricin A chain, which operate cytoplasmically by inactivation of ribosomes, are not effective to kill cells. Nevertheless, single-chain unfused Fv is useful, e.g., for imaging or radioimmunotherapy, and bispecific single-chain Fv fusion proteins of various designs, i.e., that have two distinct binding sites on the same polypeptide chain, can be used to target via the two antigens for which the molecule is specific. For example, a bispecific single-chain antibody may have specificity for both the c-erbB-2 and CD3 antigens, the latter of which is present on cytotoxic lymphocytes (CTLs). This bispecific molecule could thus mediate antibody dependent cellular cytotoxicity (ADCC) that results in CTL-induced lysis of tumor cells. Similar results could be obtained using a bispecific single-chain Fv specific for c-erbB-2 and the Fcγ receptor type I or II. Other bispecific sFv formulations include domains with c-erbB-2 specificity paired with a growth factor domain specific for hormone or growth factor receptors, such as receptors for transferrin or epidermal growth factor (EGF).

Brief Description of the Drawings

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings.

FIG. 1A is a schematic drawing of a DNA construct encoding an sFv of the invention, which shows the V_H and V_L encoding domains and the linker region; FIG. 1B is a schematic drawing of the structure of Fv illustrating V_H and V_L domains, each of which comprises three complementarity determining regions (CDRs) and four framework regions (FRs) for monoclonal 520C9, a well known and characterized murine monoclonal antibody specific for c-erbB-2;

FIGS. 2A-2E are schematic representations of embodiments of the invention, each of which comprises a biosynthetic single-chain Fv polypeptide which recognizes a c-erbB-2-related antigen: FIG. 2A is an sFv having a pendant leader sequence, FIG. 2B is an sFv-toxin (or other ancillary protein) construct, and FIG. 2C is a bivalent or bispecific sFv construct; FIG. 2D is a bivalent sFv having a pendant protein attached to the carboxyl-terminal end; FIG. 2E is a bivalent sFv having pendant proteins attached to both amino- and carboxyl-terminal ends.

FIG. 3 is a diagrammatic representation of the construction of a plasmid encoding the 520C9 sFv-ricin A fused immunotoxin gene; and

FIG. 4 is a graphic representation of the results of a competition assay comparing the c-erbB-2 binding activity of the 520C9 monoclonal antibody (specific for c-erbB-2), an Fab fragment of that monoclonal antibody (filled dots), and different affinity purified

fractions of the single-chain-Fv binding site for c-erbB-2 constructed from the variable regions of the 520C9 monoclonal antibody (sFv whole sample (+), sFv bound and eluted from a column of immobilized extracellular domain of C-erbB-2 (squares) and sFv flow-through (unbound, *)).

Detailed Description of the Invention

Disclosed are single-chain Fv's and sFv fusion proteins having affinity for a c-erbB-2-related antigen expressed at high levels on breast and ovarian cancer cells and on other tumor cells as well, in certain other forms of cancer. The polypeptides are characterized by one or more sequences of amino acids constituting a region which behaves as a biosynthetic antibody binding site. As shown in FIG. 1, the sites comprise heavy chain variable region (V_H) 10, light chain variable region (V_L) 14 single chains wherein V_H 10 and V_L 14 are attached by polypeptide linker 12. The binding domains include CDRs 2, 4, 6 and 2', 4', 6' from immunoglobulin molecules able to bind a c-erbB-2-related tumor antigen linked to FRs 32, 34, 36, 38 and 32', 34', 36' 38' which may be derived from a separate immunoglobulin. As shown in FIGS. 2A, 2B, and 2C, the BABS single polypeptide chains (V_H 10, V_L 14 and linker 12) may also include remotely detectable moieties and/or other polypeptide sequences 16, 18, or 22, which function e.g., as an enzyme, toxin, binding site, or site of attachment to an immobilization matrix or radioactive atom. Also disclosed are methods for producing the proteins and methods of their use.

The single-chain Fv polypeptides of the invention are biosynthetic in the sense that they are synthesized and recloned in a cellular host made to express a protein encoded by a plasmid which includes genetic sequence based in part on synthetic DNA, that is, a recombinant DNA made from ligation of plural, chemically synthesized and recloned oligonucleotides, or by ligation of fragments of DNA derived from the genome of a hybridoma, mature B cell clone, or a cDNA library derived from such natural sources. The

proteins of the invention are properly characterized as "antibody binding sites" in that these synthetic single polypeptide chains are able to refold into a 3-dimensional conformation designed specifically to have affinity for a preselected c-erbB-2 or related tumor antigen. Single-chain Fv's may be produced as described in PCT application US88/01737, which corresponds to USSN 342,449, filed February 6, 1989, and claims priority from USSN 052,800, filed May 21, 1987, assigned to Creative BioMolecules, Inc., hereby incorporated by reference. The polypeptides of the invention are antibody-like in that their structure is patterned after regions of native antibodies known to be responsible for c-erbB-2-related antigen recognition.

More specifically, the structure of these biosynthetic antibody binding sites (BABS) in the region which imparts the binding properties to the protein, is analogous to the Fv region of a natural antibody to a c-erbB-2 or related antigen. It includes a series of regions consisting of amino acids defining at least three polypeptide segments which together form the tertiary molecular structure responsible for affinity and binding. The CDRs are held in appropriate conformation by polypeptide segments analogous to the framework regions of the Fv fragment of natural antibodies.

The CDR and FR polypeptide segments are designed empirically based on sequence analysis of the Fv region of preexisting antibodies, such as those described in U.S. Patent No. 4,753,894, herein incorporated by reference, or of the DNA encoding such antibody molecules.

One such antibody, 520C9, is a murine monoclonal antibody that is known to react with an antigen expressed by the human breast cancer cell line SK-Br-3 (U.S. Patent 4,753,894). The antigen is an approximately 200 kD acidic glycoprotein that has an isoelectric point of 5.3, and is present at about 5 million copies per cell. The association constant measured using radiolabelled antibody is approximately $4.6 \times 10^{8}\ M^{-1}$.

In one embodiment, the amino acid sequences constituting the FRs of the single polypeptide chains are analogous to the FR sequences of a first preexisting antibody, for example, a human IgG. The amino acid sequences constituting the CDRs are analogous to the sequences from a second, different preexisting antibody, for example, the CDRs of a rodent or human IgG which recognizes c-erbB-2 or related antigens expressed on the surface of ovarian and breast tumor cells. Alternatively, the CDRs and FRs may be copied in their entirety from a single preexisting antibody from a cell line which may be unstable or, difficult to culture; e.g., an sFv-producing cell line that is based upon a murine, mouse/human, or human monoclonal antibody-secreting cell line.

Practice of the invention enables the design and biosynthesis of various reagents, all of which are characterized by a region having affinity for a preselected c-erbB-2 or related antigen. Other regions of the biosynthetic protein are designed with the particular planned utility of the protein in mind. Thus, if the reagent is designed for intravascular use in mammals, the FRs may include amino acid sequences that are similar or identical to at least a portion of the FR amino acids of antibodies native to that

mammalian species. On the other hand, the amino acid sequences that include the CDRs may be analogous to a portion of the amino acid sequences from the hypervariable region (and certain flanking amino acids) of an antibody having a known affinity and specificity for a c-erbB-2 or related antigen that is from, e.g., a mouse or rat, or a specific human antibody or immunoglobulin.

Other sections of native immunoglobulin protein structure, e.g., C_H and C_L, need not be present and normally are intentionally omitted from the biosynthetic proteins of this invention. However, the single polypeptide chains of the invention may include additional polypeptide regions defining a leader sequence or a second polypeptide chain that is bioactive, e.g., a cytokine, toxin, ligand, hormone, immunoglobulin domain(s), or enzyme, or a site onto which a toxin, drug, or a remotely detectable moiety, e.g., a radionuclide, can be attached.

One useful toxin is ricin, an enzyme from the castor bean that is highly toxic, or the portion of ricin that confers toxicity. At concentrations as low as 1 ng/ml ricin efficiently inhibits the growth of cells in culture. The ricin A chain has a molecular weight of about 30,000 and is glycosylated. The ricin B chain has a larger size (about 34,000 molecular weight) and is also glycosylated. The B chain contains two galactose binding sites, one in each of the two domains in the folded subunit. The crystallographic structure for ricin shows the backbone tracing of the A chain. There is a cleft, which is probably the active site, that runs diagonally across the molecule. Also present is a mixture of α-helix, ß-structure, and irregular structure in the molecule.

The A chain enzymatically inactivates the 60S ribosomal subunit of eucaryotic ribosomes. The B chain binds to galactose-based carbohydrate residues on the surfaces of cells. It appears to be necessary to bind the toxin to the cell surface, and also facilitates and participates in the mechanics of entry of the toxin into the cell. Because all cells have galactose-containing cell surface receptors, ricin inhibits all types of mammalian cells with nearly the same efficiency.

Ricin A chain and ricin B chain are encoded by a gene that specifies both the A and B chains. The polypeptide synthesized from the mRNA transcribed from the gene contains A chain sequences linked to B chain sequences by a 'J' (for joining) peptide. The J peptide fragment is removed by post-translational modification to release the A and B chains. However, A and B chains are still held together by the interchain disulfide bond. The preferred form of ricin is recombinant A chain as it is totally free of B chain and, when expressed in E. coli, is unglycosylated and thus cleared from the blood more slowly than the gycosylated form. The specific activity of the recombinant ricin A chain against ribosomes and that of native A chain isolated from castor bean ricin are equivalent. An amino acid sequence and corresponding nucleic acid sequence of ricin A chain is set forth in the Sequence Listing as SEQ ID NOS:7 and 8.

Recombinant ricin A chain, plant-derived ricin A chain, deglycosylated ricin A chain, or derivatives thereof, can be targeted to a cell expressing a c-erbB-2 or related antigen by the single-chain Fv polypeptide of the present invention. To do this, the sFv may be chemically crosslinked to ricin A chain or

an active analog thereof, or in a preferred embodiment a single-chain Fv-ricin A chain immunotoxin may be formed by fusing the single-chain Fv polypeptide to one or more ricin A chains through the corresponding gene fusion. By replacing the B chain of ricin with an antibody binding site to c-erbB-2 or related antigens, the A chain is guided to such antigens on the cell surface. In this way the selective killing of tumor cells expressing these antigens can be achieved. This selectivity has been demonstrated in many cases against cells grown in culture. It depends on the presence or absence of antigens on the surface of the cells to which the immunotoxin is directed.

The invention includes the use of humanized single-chain-Fv binding sites as part of imaging methods and tumor therapies. The proteins may be administered by intravenous or intramuscular injection. Effective dosages for the single-chain Fv constructs in antitumor therapies or in effective tumor imaging can be determined by routine experimentation, keeping in mind the objective of the treatment.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions. In all cases, the form must be sterile and must be fluid so as to be easily administered by syringe. It must be stable under the conditions of manufacture and storage, and must be preserved against the contaminating action of microorganisms. This may, for example, be achieved by filtration through a sterile 0.22 micron filter and/or lyophilization followed by sterilization with a gamma ray source.

Sterile injectable solutions are prepared by incorporating the single chain constructs of the invention in the required amount in the appropriate

solvent, such as sodium phosphate-buffered saline, followed by filter sterilization. As used herein, "a physiologically acceptable carrier" includes any and all solvents, dispersion media, antibacterial and antifungal agents that are non-toxic to humans, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. The media or agent must be compatible with maintenance of proper conformation of the single polypeptide chains, and its use in the therapeutic compositions. Supplementary active ingredients can also be incorporated into the compositions.

A bispecific single-chain Fv could also be fused to a toxin. For example, a bispecific sFv construct with specificity for c-erbB-2 and the transferrin receptor, a target that is rapidly internalized, would be an effective cytolytic agent due to internalization of the transferrin receptor/sFv-toxin complex. An sFv fusion protein may also include multiple protein domains on the same polypeptide chain, e.g., EGF-sFv-ricin A, where the EGF domain promotes internalization of toxin upon binding of sFv through interaction with the EGF receptor.

The single polypeptide chains of the invention can be labelled with radioisotopes such as Iodine-131, Indium-111, and Technetium-99m, for example. Beta emitters such as Technetium-99m and Indium-111 are preferred because they are detectable with a gamma camera and have favorable half-lives for imaging *in vivo*. The single polypeptide chains can be labelled, for example, with radioactive atoms and as Yttrium-90, Technetium-99m, or Indium-111 via a conjugated metal chelator (see, e.g., Khaw et al. (1980) Science 209:295; Gansow et al., U.S. Patent No. 4,472,509;

Hnatowich, U.S. Patent No. 4,479,930), or by other standard means of isotope linkage to proteins known to those with skill in the art.

The invention thus provides intact binding sites for c-erbB-2 or related antigens that are analogous to V_H-V_L dimers linked by a polypeptide sequence to form a composite $(V_H\text{-linker-}V_L)_n$ or $(V_L\text{-linker-}V_H)_n$ polypeptide, where n is equal to or greater than 1, which is essentially free of the remainder of the antibody molecule, and which may include a detectable moiety or a third polypeptide sequence linked to each V_H or V_L.

FIGs. 2A-2E illustrate examples of protein structures embodying the invention that can be produced by following the teaching disclosed herein. All are characterized by at least one biosynthetic sFv single chain segment defining a binding site, and containing amino acid sequences including CDRs and FRs, often derived from different immunoglobulins, or sequences homologous to a portion of CDRs and FRs from different immunoglobulins.

FIG. 2A depicts single polypeptide chain sFv 100 comprising polypeptide 10 having an amino acid sequence analogous to the heavy chain variable region (V_H) of a given anti-c-erbB-2 monoclonal antibody, bound through its carboxyl end to polypeptide linker 12, which in turn is bound to polypeptide 14 having an amino acid sequence analogous to the light chain variable region (V_L) of the anti-c-erbB-2 monoclonal. Of course, the light and heavy chain domains may be in reverse order. Linker 12 should be at least long enough (e.g., about 10 to 15 amino acids or about 40 Angstroms) to permit chains 10 and 14 to assume their proper conformation and interdomain relationship.

Linker 12 may include an amino acid sequence homologous to a sequence identified as "self" by the species into which it will be introduced, if drug use is intended. Unstructured, hydrophilic amino acid sequences are preferred. Such linker sequences are set forth in the Sequence Listing as amino acid residue numbers 116 through 135 in SEQ ID NOS:3, 4, 5, and 6, which include part of the 16 amino acid linker sequences set forth in the Sequence Listing SEQ ID NOS:12 and 14.

Other proteins or polypeptides may be attached to either the amino or carboxyl terminus of protein of the type illustrated in FIG. 2A. As an example, leader sequence 16 is shown extending from the amino terminal end of V_H domain 10.

FIG. 2B depicts another type of reagent 200 including a single polypeptide chain 100 and a pendant protein 18. Attached to the carboxyl end of the polypeptide chain 100 (which includes the FR and CDR sequences constituting an immunoglobulin binding site) is a pendant protein 18 consisting of, for example, a toxin or toxic fragment thereof, binding protein, enzyme or active enzyme fragment, or site of attachment for an imaging agent (e.g., to chelate a radioactive ion such as Indium-111).

FIG. 2C illustrates single chain polypeptide 300 including second single chain polypeptide 110 of the invention having the same or different specificity and connected via peptide linker 22 to the first single polypeptide chain 100.

FIG. 2D illustrates single chain polypeptide 400 which includes single polypeptide chains 110 and 100 linked together by linker 22, and pendant protein 18 attached to the carboxyl end of chain 110.

FIG. 2E illustrates single polypeptide chain 500 which includes chain 400 of Fig. 2D and pendant protein 20 (EGF) attached to the amino terminus of chain 400.

As is evident from Figs. 2A-E, single chain proteins of the invention may resemble beads on a string by including multiple biosynthetic binding sites, each binding site having unique specificity, or repeated sites of the same specificity to increase the avidity of the protein. As is evidenced from the foregoing, the invention provides a large family of reagents comprising proteins, at least a portion of which defines a binding site patterned after the variable region or regions of immunoglobulins to c-erbB-2 or related antigens.

The single chain polypeptides of the invention are designed at the DNA level. The synthetic DNAs are then expressed in a suitable host system, and the expressed proteins are collected and renatured if necessary.

The ability to design the single polypeptide chains of the invention depends on the ability to identify monoclonal antibodies of interest, and then to determine the sequence of the amino acids in the variable region of these antibodies, or the DNA sequence encoding them. Hybridoma technology enables production of cell lines secreting antibody to essentially any desired substance that elicits an immune response. For example, U.S. Patent No. 4,753,894 describes some monoclonal antibodies of interest which recognize c-erbB-2 related antigens on breast cancer cells, and explains how such antibodies were obtained. One monoclonal antibody that is particularly useful for this purpose is 520C9 (Bjorn et al. (1985) Cancer Res. 45:124-1221; U.S. Patent

No. 4,753,894). This antibody specifically recognizes the c-erbB-2 antigen expressed on the surface of various tumor cell lines, and exhibits very little binding to normal tissues. Alternative sources of sFv sequences with the desired specificity can take advantage of phage antibody and combinatorial library methodology. Such sequences would be based on cDNA from mice which were preimmunized with tumor cell membranes or c-erb-B-2 or c-erbB-2-related antigenic fragments or peptides. (See, e.g., Clackson et al, Nature 352 624-628 (1991))

The process of designing DNA that encodes the single polypeptide chain of interest can be accomplished as follows. RNA encoding the light and heavy chains of the desired immunoglobulin can be obtained from the cytoplasm of the hyridoma producing the immunoglobulin. The mRNA can be used to prepare the cDNA for subsequent isolation of V_H and V_L genes by PCR methodology known in the art (Sambrook et al., eds., Molecular Cloning, 1989, Cold Spring Harbor Laboratories Press, NY). The N-terminal amino acid sequence of H and L chain may be independently determined by automated Edman sequencing; if necessary, further stretches of the CDRs and flanking FRs can be determined by amino acid sequencing of the H and L chain V region fragments. Such sequence analysis is now conducted routinely. This knowledge permits one to design synthetic primers for isolation of V_H and V_L genes from hybridoma cells that make monoclonal antibodies known to bind the c-erbB-2 or related antigen. These V genes will encode the Fv region that binds c-erbB-2 in the parent antibody.

Still another approach involves the design and construction of synthetic V genes that will encode an Fv binding site specific for c-erbB-2 or related

receptors. For example, with the help of a computer program such as, for example, Compugene, and known variable region DNA sequences, one may design and directly synthesize native or near-native FR sequences from a first antibody molecule, and CDR sequences from a second antibody molecule. The V_H and V_L sequences described above are linked together directly via an amino acid chain or linker connecting the C-terminus of one chain with the N-terminus of the other.

These genes, once synthesized, may be cloned with or without additional DNA sequences coding for, e.g., a leader peptide which facilitates secretion or intracellular stability of a fusion polypeptide, or a leader or trailing sequence coding for a second polypeptide. The genes then can be expressed directly in an appropriate host cell.

By directly sequencing an antibody to a c-erbB-2 or related antigen, or obtaining the sequence from the literature, in view of this disclosure, one skilled in the art can produce a single chain Fv comprising any desired CDR and FR. For example, using the DNA sequence for the 520C9 monoclonal antibody set forth in the Sequence Listing as SEQ ID NO:3, a single chain polypeptide can be produced having a binding affinity for a c-erbB-2 related antigen. Expressed sequences may be tested for binding and empirically refined by exchanging selected amino acids in relatively conserved regions, based on observation of trends in amino acid sequence data and/or computer modeling techniques. Significant flexibility in V_H and V_L design is possible because alterations in amino acid sequences may be made at the DNA level.

Accordingly, the construction of DNAs encoding the single-chain Fv and sFv fusion proteins of the

invention can be done using known techniques involving the use of various restriction enzymes which make sequence-specific cuts in DNA to produce blunt ends or cohesive ends, DNA ligases, techniques enabling enzymatic addition of sticky ends to blunt-ended DNA, construction of synthetic DNAs by assembly of short or medium length oligonucleotides, cDNA synthesis techniques, and synthetic probes for isolating immunoglobulin genes. Various promoter sequences and other regulatory RNA sequences used in achieving expression, and various type of host cells are also known and available. Conventional transfection techniques, and equally conventional techniques for cloning and subcloning DNA are useful in the practice of this invention and known to those skilled in the art. Various types of vectors may be used such as plasmids and viruses including animal viruses and bacteriophages. The vectors may exploit various marker genes which impart to a successfully transfected cell a detectable phenotypic property that can be used to identify which of a family of clones has successfully incorporated the recombinant DNA of the vector.

Of course, the processes for manipulating, amplifying, and recombining DNA which encode amino acid sequences of interest are generally well known in the art, and therefore, not described in detail herein. Methods of identifying the isolated V genes encoding antibody Fv regions of interest are well understood, and described in the patent and other literature. In general, the methods involve selecting genetic material coding for amino acid sequences which define the CDRs and FRs of interest upon reverse transcription, according to the genetic code.

One method of obtaining DNA encoding the single-chain Fv disclosed herein is by assembly of synthetic oligonucleotides produced in a conventional, automated, polynucleotide synthesizer followed by ligation with appropriate ligases. For example, overlapping, complementary DNA fragments comprising 15 bases may be synthesized semi-manually using phosphoramidite chemistry, with end segments left unphosphorylated to prevent polymerization during ligation. One end of the synthetic DNA is left with a "sticky end" corresponding to the site of action of a particular restriction endonuclease, and the other end is left with an end corresponding to the site of action of another restriction endonuclease. Alternatively, this approach can be fully automated. The DNA encoding the single chain polypeptides may be created by synthesizing longer single strand fragments (e.g., 50-100 nucleotides long) in, for example, a Biosearch oligonucleotide synthesizer, and then ligating the fragments.

Additional nucleotide sequences encoding, for example, constant region amino acids or a bioactive molecule may also be linked to the gene sequences to produce a bifunctional protein.

For example, the synthetic genes and DNA fragments designed as described above may be produced by assembly of chemically synthesized oligonucleotides. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer (TBE). The DNA is then electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

The blocks or the pairs of longer oligonucleotides may be cloned in E. coli using a suitable cloning vector, e.g., pUC. Initially, this vector may be altered by single-strand mutagenesis to eliminate residual six base altered sites. For example, V_H may be synthesized and cloned into pUC as five primary blocks spanning the following restriction sites: (1) EcoRI to first NarI site; (2) first NarI to XbaI; (3) XbaI to SalI; (4) SalI to NcoI; and (5) NcoI to BamHI. These cloned fragments may then be isolated and assembled in several three-fragment ligations and cloning steps into the pUC8 plasmid. Desired ligations, selected by PAGE, are then transformed into, for example, E. coli strain JM83, and plated onto LB Ampicillin + Xgal plates according to standard procedures. The gene sequence may be confirmed by supercoil sequencing after cloning, or after subcloning into M13 via the dideoxy method of Sanger (Molecular Cloning, 1989, Sambrook et al., eds, 2d ed., Vol. 2, Cold Spring Harbor Laboratory Press, NY).

The engineered genes can be expressed in appropriate prokaryotic hosts such as various strains of E. coli, and in eucaryotic hosts such as Chinese hamster ovary cells (CHO), mouse myeloma, hybridoma, transfectoma, and human myeloma cells.

If the gene is to be expressed in E. coli, it may first be cloned into an expression vector. This is accomplished by positioning the engineered gene downstream from a promoter sequence such as Trp or Tac, and a gene coding for a leader polypeptide such as fragment B (FB) of staphylococcal protein A. The resulting expressed fusion protein accumulates in refractile bodies in the cytoplasm of the cells, and may be harvested after disruption of the cells by

French press or sonication. The refractile bodies are solubilized, and the expressed fusion proteins are cleaved and refolded by the methods already established for many other recombinant proteins (Huston et al, 1988, supra) or, for direct expression methods, there is no leader and the inclusion bodies may be refolded without cleavage (Huston et al, 1991, Methods in Enzymology, vol 203, pp 46-88).

For example, subsequent proteolytic cleavage of the isolated sFv from their leader sequence fusions can be performed to yield free sFvs, which can be renatured to obtain an intact biosynthetic, hybrid antibody binding site. The cleavage site preferably is immediately adjacent the sFv polypeptide and includes one amino acid or a sequence of amino acids exclusive of any one amino acid or amino acid sequence found in the amino acid structure of the single polypeptide chain.

The cleavage site preferably is designed for specific cleavage by a selected agent. Endopeptidases are preferred, although non-enzymatic (chemical) cleavage agents may be used. Many useful cleavage agents, for instance, cyanogen bromide, dilute acid, trypsin, <u>Staphylococcus</u> <u>aureus</u> V-8 protease, post-proline cleaving enzyme, blood coagulation Factor Xa, enterokinase, and renin, recognize and preferentially or exclusively cleave at particular cleavage sites. One currently preferred peptide sequence cleavage agent is V-8 protease. The currently preferred cleavage site is at a Glu residue. Other useful enzymes recognize multiple residues as a cleavage site, e.g., factor Xa (Ile-Glu-Gly-Arg) or enterokinase (Asp-Asp-Asp-Asp-Lys). Dilute acid preferentially leaves the peptide bond between Asp-Pro residues, and CNBr in acid cleaves after Met, unless it is followed by Tyr.

If the engineered gene is to be expressed in eucaryotic hybridoma cells, the conventional expression system for immunoglobulins, it is first inserted into an expression vector containing, for example, the immunoglobulin promoter, a secretion signal, immunoglobulin enhancers, and various introns. This plasmid may also contain sequences encoding another polypeptide such as all or part of a constant region, enabling an entire part of a heavy or light chain to be expressed, or at least part of a toxin, enzyme, cytokine, or hormone. The gene is transfected into myeloma cells via established electroporation or protoplast fusion methods. Cells so transfected may then express V_H-linker-V_L or V_L-linker-V_H single-chain Fv polypeptides, each of which may be attached in the various ways discussed above to a protein domain having another function (e.g., cytotoxicity).

For construction of a single contiguous chain of amino acids specifying multiple binding sites, restriction sites at the boundaries of DNA encoding a single binding site (i.e., V_H-linker-V_L) are utilized or created, if not already present. DNAs encoding single binding sites are ligated and cloned into shuttle plasmids, from which they may be further assembled and cloned into the expression plasmid. The order of domains will be varied and spacers between the domains provide flexibility needed for independent folding of the domains. The optimal architecture with respect to expression levels, refolding and functional activity will be determined empirically. To create bivalent sFv's, for example, the stop codon in the gene encoding the first binding site is changed to an open reading frame, and several glycine plus serine codons including a restriction site such as BamHI (encoding

Gly-Ser) or XhoI (encoding Gly-Ser-Ser) are put in place. The second sFv gene is modified similarly at its 5' end, receiving the same restriction site in the same reading frame. The genes are combined at this site to produce the bivalent sFv gene.

Linkers connecting the C-terminus of one domain to the N-terminus of the next generally comprise hydrophilic amino acids which assume an unstructured configuration in physiological solutions and preferably are free of residues having large side groups which might interfere with proper folding of the V_H, V_L, or pendant chains. One useful linker has the amino acid sequence $[(Gly)_4Ser]_3$ (see SEQ ID NOS:5 and 6, residue numbers 121-135). One currently preferred linker has the amino acid sequence comprising 2 or 3 repeats of $[(Ser)_4Gly]$, such as $[(Ser)_4Gly]_2$ and $[(Ser)_4Gly]_3$ (see SEQ ID NOS:3 and 4).

The invention is illustrated further by the following non-limiting Examples.

EXAMPLES

1. Antibodies to c-erbB-2 Related Antigens

Monoclonal antibodies against breast cancer have been developed using human breast cancer cells or membrane extracts of the cells for immunizing mice, as described in Frankel et al. (1985) J. Biol. Resp. Modif. 4:273-286, hereby incorporated by reference. Hybridomas have been made and selected for production of antibodies using a panel of normal and breast cancer cells. A panel of eight normal tissue membranes, a fibroblast cell line, and frozen sections of breast cancer tissues were used in the screening. Candidates that passed the first screening were further tested on 16 normal tissue sections, 5 normal blood cell types,

11 nonbreast neoplasm sections, 21 breast cancer sections, and 14 breast cancer cell lines. From this selection, 127 antibodies were selected. Irrelevant antibodies and nonbreast cancer cell lines were used in control experiments.

Useful monoclonal antibodies were found to include 520C9, 454C11 (A.T.C.C. Nos. HB8696 and HB8484, respectively) and 741F8. Antibodies identified as selective for breast cancer in this screen reacted against five different antigens. The sizes of the antigens that the antibodies recognize: 200 kD; a series of proteins that are probably degradation products with Mr's of 200 kD, 93kD, 60 kD, and 37 kD; 180 kD (transferrin receptor); 42 kD; and 55 kD, respectively. Of the antibodies directed against the five classes of antigens, the most specific are the ones directed against the 200 kD antigen, 520C9 being a representative antibody for that antigen class. 520C9 reacts with fewer breast cancer tissues (about 20-70% depending on the assay conditions) and it reacts with the fewest normal tissues of any of the antibodies. 520C9 reacts with kidney tubules (as do many monoclonal antibodies), but not pancreas, esophagus, lung, colon, stomach, brain, tonsil, liver, heart, ovary, skin, bone, uterus, bladder, or normal breast among some of the tissues tested.

2. <u>Preparation of cDNA Library Encoding 520C9 Antibody</u>.

Polyadenylated RNA was isolated from approximately 1 x 10^8 (520C9 hybridoma) cells using the "FAST TRACK" mRNA isolation kit from Invitrogen (San Diego, CA). The presence of immunoglobulin heavy chain RNA was confirmed by Northern analysis (Molecular Cloning, 1989, Sambrook et al., eds., 2d ed., Cold

Spring Harbor Laboratory Press, NY) using a recombinant probe containing the various J regions of heavy chain genomic DNA. Using 6 μg RNA for each, cDNA was prepared using the Invitrogen cDNA synthesis system with either random and oligo dT primers. Following synthesis, the cDNA was size-selected by isolating 0.5-3.0 Kilobase (Kb) fragments following agarose gel electrophoresis. After optimizing the cDNA to vector ratio, these fragments were then ligated to the pcDNA II Invitrogen cloning vector.

3. Isolation of V_H and V_L Domains

After transformation of the bacteria with plasmid library DNA, colony hybridization was performed using antibody constant (C) region and joining (J) region probes for either light or heavy chain genes. See Orlandi, R., et al., 1989, Proc. Nat. Aca. Sci. 86:3833. The antibody constant region probe can be obtained from any of light or heavy chain nucleotide sequences from an immunoglobulin gene using known procedures. Several potential positive clones were identified for both heavy and light chain genes and, after purification by a second round of screening, these were sequenced. One clone (M207) contained the sequence of non-functional Kappa chain which has a tyrosine substituted for a conserved cysteine, and also terminates prematurely due to a 4 base deletion which causes a frame-shift mutation in the variable-J region junction. A second light chain clone (M230) contained virtually the entire 520C9 light chain gene except for the last 18 amino acids of the constant region and approximately half of the signal sequence. The 520C9 heavy chain variable region was present on a clone of approximately 1,100 base pairs (F320) which ended near the end of the CH2 domain.

4. Mutagenesis of V_H AND V_L

In order to construct the sFv, both the heavy and light chain variable regions were mutagenized to insert appropriate restriction sites (Kunkel, T.A., 1985, Proc. Nat. Acad. Sci. USA 82:1373). The heavy chain clone (F320) was mutagenized to insert a BamH1 site at the 5' end of V_H (F321). The light chain was also mutagenized simultaneously by inserting an EcoRV site at the 5' end and a PstI site with a translation stop codon at the 3' end of the variable region (M231).

5. Sequencing

cDNA clones encoding light and heavy chain were sequenced using external standard pUC primers and several specific internal primers which were prepared on the basis of the sequences obtained for the heavy chain. The nucleotide sequences were analyzed in a Genbank homology search (program Nucscan of DNA-star) to eliminate endogenous immunoglobulin genes. Translation into amino acids was checked with amino acid sequences in the NIH atlas edited by E. Kabat.

Amino acid sequences derived from 520C9 immunoglobulin confirmed the identity of these V_H and V_L cDNA clones. The heavy chain clone pF320 started 6 nucleotides upstream of the first ATG codon and extended into the CH2-encoding region, but it lacked the last nine amino acid codons of the CH2 constant domain and all of the CH3 coding region, as well as the 3' untranslated region and the poly A tail. Another short heavy chain clone containing only the CH2 and CH3 coding regions, and the poly A tail was initially assumed to represent the missing part of the 520C9 heavy chain. However, overlap between both sequences was not identical. The 520C9 clone (pF320) encodes the CH1 and CH2 domains of murine IgG1, whereas the short clone pF315 encodes the CH2 and CH3 of IgG2b.

6. Gene Design

A nucleic acid sequence encoding a composite 520C9 sFv region containing a single-chain Fv binding site which recognizes c-erbB-2 related tumor antigens was designed with the aid of Compugene software. The gene contains nucleic acid sequences encoding the V_H and V_L regions of the 520C9 antibody described above linked together with a double-stranded synthetic oligonucleotide coding for a peptide with the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 116 through 133 in SEQ ID NOS:3 and 4. This linker oligonucleotide contains helper cloning sites EcoRI and BamHI, and was designed to contain the assembly sites SacI and EcoRV near its 5′ and 3′ ends, respectively. These sites enable match-up and ligation to the 3′ and 5′ ends of 520C9 V_H and V_L, respectively, which also contain these sites (V_H-linker-V_L). However, the order of linkage to the oligonucleotide may be reversed (V_L-linker-V_H) in this or any sFv of the invention. Other restriction sites were designed into the gene to provide alternative assembly sites. A sequence encoding the FB fragment of protein A was used as a leader.

The invention also embodies a humanized single-chain Fv, i.e., containing human framework sequences and CDR sequences which specify c-erbB-2 binding, e.g., like the CDRs of the 520C9 antibody. The humanized Fv is thus capable of binding c-erbB-2 while eliciting little or no immune response when administered to a patient. A nucleic acid sequence encoding a humanized sFv may be designed and constructed as follows. Two strategies for sFv design are especially useful. A homology search in the GenBank database for the most related human framework (FR) regions may be performed

and FR regions of the sFv may be mutagenized according to sequences identified in the search to reproduce the corresponding human sequence; or information from computer modeling based on x-ray structures of model Fab fragments may be used (Amit et al., 1986, Science 233:747-753; Colman et al., 1987, Nature 326:358-363; Sheriff et al., 1987, Proc. Nat. Aca. Sci., 84:8075-8079; and Satow et al., 1986, J. Mol. Biol. 190:593-604, all of which are hereby incorporated by reference). In a preferred case, the most homologous human V_H and V_L sequences may be selected from a collection of PCR-cloned human V regions. The FRs are made synthetically and fused to CDRs to make successively more complete V regions by PCR-based ligation, until the full humanized V_L and V_H are completed. For example, a humanized sFv that is a hybrid of the murine 520C9 antibody CDRs and the human myeloma protein NEW FRs can be designed such that each variable region has the murine binding site within a human framework (FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4). The Fab NEW crystal structure (Saul et al., 1978, J. Biol. Chem. 253:585-597) also may be used to predict the location of FRs in the variable regions. Once these regions are predicted, the amino acid sequence or the corresponding nucleotide sequence of the regions may be determined, and the sequences may be synthesized and cloned into shuttle plasmids, from which they may be further assembled and cloned into an expression plasmid; alternatively, the FR sequences of the 520C9 sFv may be mutagenized directly and the changes verified by supercoil sequencing with internal primers (Chen et al., 1985, DNA 4:165-170).

7. Preparation of and Purification 520C9 sFv

A. Inclusion Body Solubilization.

The 520C9 sFv plasmid, based on a T_7 promoter and vector, was made by direct expression in E. coli of the fused gene sequence set forth in the Sequence Listing as SEQ. ID NO:3. Inclusion bodies (15.8 g) from a 2.0 liter fermentation were washed with 25 mM Tris, 10 mM EDTA, pH 8.0 (TE), plus 1 M guanidine hydrochloride (GuHCl). The inclusion bodies were solubilized in TE, 6 M GuHCl, 10 mM dithiothreitol (DTT), pH 9.0, and yielded 3825 A_{280} units of material. This material was ethanol precipitated, washed with TE, 3M urea, then resuspended in TE, 8M urea, 10 mM DTT, pH 8.0. This precipitation step prepared the protein for ion exchange purification of the denatured sFv.

B. Ion Exchange Chromatography

The solubilized inclusion bodies were subjected to ion exchange chromatography in an effort to remove contaminating nucleic acids and E. coli proteins before renaturation of the sFv. The solubilized inclusion bodies in 8M urea were diluted with TE to a final urea concentration of 6M, then passed through 100 ml of DEAE-Sepharose Fast Flow in a radial flow column. The sFv was recovered in the unbound fraction (69% of the starting sample).

The pH of this sFv solution (A_{280} = 5.7; 290 ml) was adjusted to 5.5 with 1 M acetic acid to prepare it for application to an S-Sepharose Fast Flow column. When the pH went below 6.0, however, precipitate formed in the sample. The sample was clarified; 60% of the sample was in the pellet and 40% in the supernatant. The supernatant was passed through 100 ml S-Sepharose Fast Flow and the sFv recovered in the unbound fraction. The pellet was resolubilized in TE, 6 M

GuHCl, 10 mM DTT, pH 9.0, and was also found to contain primarily sFv in a pool of 45 ml volume with an absorbance at 280 nm of 20 absorbance units. This reduced sFv pool was carried through the remaining steps of the purification.

C. Renaturation of sFv

Renaturation of the sFv was accomplished using a disulfide-restricted refolding approach, in which the disulfides were oxidized while the sFv was fully denatured, followed by removal of the denaturant and refolding. Oxidation of the sFv samples was carried out in TE, 6 M GuHCl, 1 mM oxidized glutathione (GSSG), 0.1 mM reduced glutathione (GSH), pH 9.0. The sFv was diluted into the oxidation buffer to a final protein A_{280} = 0.075 with a volume of 4000 ml and incubated overnight at room temperature. After overnight oxidation this solution was dialyzed against 10 mM sodium phosphate, 1 mM EDTA, 150 mM NaCl, 500 mM urea, pH 8.0 (PENU) [4 x (20 liters X 24 hrs)]. Low levels of activity were detected in the refolded sample.

D. Membrane Fractionation and Concentration of Active sFv

In order to remove aggregated misfolded material before any concentration step, the dialyzed refolded 520C9 sFv (5050 ml) was filtered through a 100K MWCO membrane (100,000 mol. wt. cut-off) (4 x 60 cm^2) using a Minitan ultrafiltration device (Millipore). This step required a considerable length of time (9 hours), primarily due to formation of precipitate in the retentate and membrane fouling as the protein concentration in the retentate increased. 95% of the protein in the refolded sample was retained by the 100K membranes, with 79% in the form of insoluble material. The 100K retentate had very low activity and was discarded.

The 100K filtrate contained most of the soluble sFv activity for binding c-erbB-2, and it was next concentrated using 10K MWCO membranes (10,000 mol. wt. cut-off) (4 x 60 cm^2) in the Minitan, to a volume of 100 ml (50X). This material was further concentrated using a YM10 10K MWCO membrane in a 50 ml Amicon stirred cell to a final volume of 5.2 ml (1000X). Only a slight amount of precipitate formed during the two 10K concentration steps. The specific activity of this concentrated material was significantly increased relative to the initial dialyzed refolding.

E. Size Exclusion Chromatography of Concentrated sFv

When refolded sFv was fractionated by size exclusion chromatography, all 520C9 sFv activity was determined to elut at the position of folded monomer. In order to enrich for active monomers, the 1000X concentrated sFv sample was fractionated on a Sephacryl S-200 HR column (2.5 x 40 cm) in PBSA (2.7 mM KCl, 1.1 mM KH_2PO_4, 138 mM NaCl, 8.1 mM $Na_2HPO_4 \cdot 7H_2O$, 0.02% NaN_3) + 0.5 M urea. The elution profile of the column and SDS-PAGE analysis of the fractions showed two sFv monomer peaks. The two sFv monomer peak fractions were pooled (10 ml total) and displayed c-erbB-2 binding activity in competition assays.

F. Affinity Purification of 520C9 sFv

The extracellular domain of (ECD) c-erbB-2 was expressed in bacculovirus-infected insect cells. This protein (ECD c-erbB-2) was immobilized on an agarose affinity matrix. The sFv monomer peak was dialyzed against PBSA to remove the urea and then applied to a 0.7 x 4.5 cm ECD c-erbB-2-agarose affinity column in PBSA. The column was washed to baseline A_{280}, then eluted with PBSA + 3 M LiCl, pH = 6.1. The peak

fractions were pooled (4 ml) and dialyzed against PBSA to remove the LiCl. 72 μg of purified sFv was obtained from 750 μg of S-200 monomer fractions. Activity measurements on the column fractions were determined by a competitive assay. Briefly, sFv affinity purification fractions and HRP-conjugated 520C9 Fab fragments were allowed to compete for binding to SK-BR-3 membranes. Successful binding of the sFv preparation prevented the HRP-52069 Fab fragment from binding to the membranes, thus also reducing or preventing utilization of the HRP substrate, and no color development (see below for details of competition assay). The results showed that virtually all of the sFv activity was bound by the column and was recovered in the eluted peak (Figure 4). As expected, the specific activity of the eluted peak was increased relative to the column sample, and appeared to be essentially the same as the parent Fab control, within the experimental error of these measurements.

9. Yield After Purification.

Table I shows the yield of various 520C9 preparations during the purification process. Protein concentration (μg/ml) was determined by the BioRad protein assay. Under "Total Yield", 300 AU denatured sFv stock represents 3.15 g inclusion bodies from 0.4 liters fermentation. The oxidation buffer was 25 mM Tris, 10 mM EDTA, 6 M GdnHCl, 1 MM GSSG, 0.1 mM GSH, pH 9.0. Oxidation was performed at room temperature overnight. Oxidized sample was dialyzed against 10 mM sodium phosphate, 1 mM EDTA, 150 mM NaCl, 500 mM urea, pH 8.0. All subsequent steps were carried out in this buffer, except for affinity chromatography, which was carried out in PBSA.

Table I

Sample	Volume	Protein Concentration	Total Yield	% Yield
1. Refolding III (oxidation)	4000 ml	0.075 A_{280}	300 AU	-
2. Dialyzed Refolding III	5050 ml	38 μg/ml	191.9 mg	100
3. Minitan 100K Filtrate	5000 ml	2 μg/ml	10.0 mg	5.4
4. Minitan 10K Retentate	100 ml	45 μg/ml	4.5 mg	2.3
6. YM10 10K Retentate	5.2 ml	600 μg/ml	3.1 mg	1.6
7. S-200 sFv Monomer Peak	10.0 ml	58 μg/ml	0.58 mg	0.3
8. Affinity Purified sFv	5.5 ml	13 μg/ml	0.07 mg	0.04

10. Immunotoxin Construction

The ricin A-520C9 single chain fused immunotoxin (SEQ. ID NO:7) encoding gene was constructed by isolating the gene coding for ricin A on a HindIII to BamH1 fragment from pPL229 (Cetus Corporation, Emeryville, CA) and using it upstream of the 520C9 sFv in pH777, as shown in FIG. 3. This fusion contains the 122 amino acid natural linker present between the A and B domains of ricin. However, in the original pRAP229 expression vector the codon for amino acid 268 of ricin was converted to a TAA translation stop codon so that the expression of the resulting gene produces only ricin A. Therefore, in order to remove the translation stop codon, site-directed mutagenesis was performed to remove the TAA and restore the natural serine codon. This then allows translation to continue through the entire immunotoxin gene.

In order to insert the immunotoxin back into the pPL229 and pRAP229 expression vectors, the PstI site at the end of the immunotoxin gene had to be converted to a sequence that was compatible with the BamHI site in vector. A synthetic oligonucleotide adaptor containing a BclI site nested between PstI ends was inserted. BclI and BamHI ends are compatible and can be combined into a hybrid BclI/BamHI site. Since BclI nuclease is sensitive to dam methylation, the construction first was transformed into a dam(-) E. coli strain, Gm48, in order to digest the plasmid DNA with BclI (and HindIII), then insert the entire immunotoxin gene on a HindIII/BclI fragment back into both Hind III/BamHI-digested expression vectors.

When native 520C9 IgG1 is conjugated with native ricin A chain or recombinant ricin A chain, the resulting immunotoxin is able to inhibit protein

synthesis by 50% at a concentration of about 0.4 x 10^{-9} M against SK-Br-3 cells. In addition to reacting with SK-Br-3 breast cancer cells, native 520C9 IgG1 immunotoxin also inhibits an ovarian cancer cell line, OVCAR-3, with a ID_{50} of 2.0 x 10^{-9} M.

In the ricin A-sFv fusion protein described above, ricin acts as leader for expression, i.e., is fused to the amino terminus of sFv. Following direct expression, soluble protein was shown to react with antibodies against native 520C9 Fab and also to exhibit ricin A chain enzymatic activity.

In another design, the ricin A chain is fused to the carboxy terminus of sFv. The 520C9 sFv may be secreted via the PelB signal sequence with ricin A chain attached to the C-terminus of sFv. For this construct, sequences encoding the PelB-signal sequence, sFv, and ricin are joined in a bluescript plasmid via a HindIII site directly following sFv (in our expression plasmids) and the HindIII site preceding the ricin gene, in a three part assembly (RI-HindIII-BamHI). A new PstI site following the ricin gene is obtained via the Bluescript polylinker. Mutagenesis of this DNA removes the stop codon and the original PstI site at the end of sFv, and places several serine residues between the sFv and ricin genes. This new gene fusion, PelB signal sequence/sFv/ricin A, can be inserted into expression vectors as an EcoRI/PstI fragment.

In another design, the pseudomonas exotoxin fragment analogous to ricin A chain, PE40, is fused to the carboxy terminus of the anti-c-erbB-2 741F8 sFv (Seq ID NOS: 15 and 16). The resulting 741F8 sFv-PE40 is a single-chain Fv-toxin fusion protein, which was constructed with an 18 residue short FB leader which initially was left on the protein. E. coli expression

of this protein produced inclusion bodies that were refolded in a 3 M urea glutathione/redox buffer. The resulting sFv-PE40 was shown to specifically kill c-erbB-2 bearing cells in culture more fully and with apparently better cytotoxicity than the corresponding crosslinked immunotoxin. The sFv-toxin protein, as well as the 741F8 sFv, can be made in good yields by these procedures, and may be used as therapeutic and diagnostic agents for tumors bearing the c-erbB-2 or related antigens, such as breast and ovarian cancer.

11. Assays

A. Competition ELISA

SK-Br-3 extract is prepared as a source of c-erbB-2 antigen as follows. SK-Br-3 breast cancer cells (Ring et al. 1989, Cancer Research 49:3070-3080), are grown to near confluence in Iscove's medium (Gibco BRL, Gaithersburg, Md.) plus 5% fetal bovine serum and 2 mM glutamine. The medium is aspirated, and the cells are rinsed with 10 ml fetal bovine serum (FBS) plus calcium and magnesium. The cells are scraped off with a rubber policeman into 10 ml FBS plus calcium and magnesium, and the flask is rinsed out with another 5 ml of this buffer. The cells are then centrifuged at 100 rpm. The supernate is aspirated off, and the cells are resuspended at 10^7 cells/ml in 10 mM NaCl, 0.5% NP40, pH 8 (TNN buffer), and are pipetted up and down to dissolve the pellet. The solution is then centrifuged at 1000 rpm to remove nuclei and other insoluble debris. The extract is filtered through 0.45 Millex HA and 0.2 Millex Gv filters. The TNN extract is stored as aliquots in Wheaton freezing vials at -70°C.

A fresh vial of SK-Br-3 TNN extract is thawed and diluted 200-fold into deionized water. Immediately thereafter, 40ug per well are added to a Dynatech PVC

96 well plak, which is allowed to sit overnight in a 37°C dry incubator. The plates are washed four times in phosphate buffered saline (PBS), 1% skim milk, 0.05% Tween 20.

The non-specific binding sites are blocked as follows. When the plate is dry, 100 ug per well PBS is added containing 1% skim milk, and the incubation allowed to proceed for one hour at room temperature.

The single-chain Fv test samples and standard 520C9 whole antibody dilutions are then added as follows. 520C9 antibody and test samples are diluted in dilution buffer (PBS + 1% skim milk) in serial two-fold steps, initially at 50ug/ml and making at least 10 dilutions for 520C9 standards. A control containing only dilution buffer is included. The diluted samples and standards are added at 50ul per well and incubated for 30 minutes at room temperature.

The 520C9-horseradish peroxidase (HRP) probe is added as follows. 520C9-HRP conjugate (Zymed Labs., South San Francisco, California) is diluted to 14 ug/ml with 1% skim milk in dilution buffer. The optimum dilutions must be determined for each new batch of peroxidase conjugate without removing the previous steps. 20 ul per well of probe was added and incubated for one hour at room temperature. The plate is then washed four times in PBS. The peroxidase substrate is then added. The substrate solution should be made fresh for each use by diluting tetramethyl benzidine stock (TMB; 2mg/ml in 100% ethanol) 1:20 and 3% hydrogen peroxide stock 1:2200 in substrate buffer (10mM sodium acetate, 10mM Na, EDTA, pH 5.0). This is incubated for 30 minutes at room temperature. The wells are then quenched with 100 ul per well 0.8 M H_2SO_4 and the absorbance at 150 nm read.

FIG. 4 compares the binding ability of the parent refolded but unpurified 520C9 monoclonal antibody, 520C9 Fab fragments, and the 520C9 sFv single-chain binding site after binding and elution from an affinity column (eluted) or the unbound flow through fraction (passed). In Fig. 4, the fully purified 520C9 sFv exhibits an affinity for c-erbB-2 that is indistinguishable from the parent monoclonal antibody, within the error of measuring protein concentration.

B. <u>In vivo testing</u>

Immunotoxins that are strong inhibitors of protein synthesis against breast cancer cells grown in culture may be tested for their <u>in vivo</u> efficacy. The <u>in vivo</u> assay is typically done in a nude mouse model using xenografts of human MX-1 breast cancer cells. Mice are injected with either PBS (control) or different concentrations of sFv-toxin immunotoxin, and a concentration-dependent inhibition of tumor growth will be observed. It is expected that higher doses of immunotoxin will produce a better effect.

The invention may be embodied in other specific forms without departing from the spirit and scope thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalence of the claims are intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Huston, James S.
Oppermann, Hermann
Houston, L. L.
Ring, David B.

(ii) TITLE OF INVENTION: Biosynthetic Binding Protein for Cancer Marker

(iii) NUMBER OF SEQUENCES: 16

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Edmund R. Pitcher, Testa, Hurwitz, & Thibeault
(B) STREET: Exchange Place, 53 State Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Pitcher, Edmund R.
(B) REGISTRATION NUMBER: 27,829
(C) REFERENCE/DOCKET NUMBER: 2054/22

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 248-7000
(B) TELEFAX: (617) 248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4299 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4299
(D) OTHER INFORMATION: /note= "product = "c-erb-b-2""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1 5 10 15

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Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
20 25 30

CTG CGG CTC CCT GCC AGT CCC GAG ACC CAC CTG GAC ATG CTC CGC CAC 144
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
35 40 45

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Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

CTG CCC ACC AAT GCC AGC CTG TCC TTC CTG CAG GAT ATC CAG GAG GTG 240
Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
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Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

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Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

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GTC ACA GGG GCC TCC CCA GGA GGC CTG CGG GAG CTG CAG CTT CGA AGC 432
Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
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Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
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Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

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Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
180 185 190

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His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

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Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

GCC CGC TGC AAG GGG CCA CTG CCC ACT GAC TGC TGC CAT GAG CAG TGT      720
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225                 230                 235                 240

GCT GCC GGC TGC ACG GGC CCC AAG CAC TCT GAC TGC CTG GCC TGC CTC      768
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

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His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

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Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
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Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

GTG AGG GCA GTT ACC AGT GCC AAT ATC CAG GAG TTT GCT GGC TGC AAG     1104
Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

AAG ATC TTT GGG AGC CTG GCA TTT CTG CCG GAG AGC TTT GAT GGG GAC     1152
Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

CCA GCC TCC AAC ACT GCC CCG CTC CAG CCA GAG CAG CTC CAA GTG TTT     1200
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385                 390                 395                 400

GAG ACT CTG GAA GAG ATC ACA GGT TAC CTA TAC ATC TCA GCA TGG CCG     1248
Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415
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Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

GGA CGA ATT CTG CAC AAT GGC GCC TAC TCG CTG ACC CTG CAA GGG CTG        1344
Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

GGC ATC AGC TGG CTG GGG CTG CGC TCA CTG AGG GAA CTG GGC AGT GGA        1392
Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

CTG GCC CTC ATC CAC CAT AAC ACC CAC CTC TGC TTC GTG CAC ACG GTG        1440
Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

CCC TGG GAC CAG CTC TTT CGG AAC CCG CAC CAA GCT CTG CTC CAC ACT        1488
Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
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Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

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Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
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545                 550                 555                 560

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Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
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Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590

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Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605

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Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
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785 790 795 800

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835 840 845

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850 855 860

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885 890 895

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900 905 910

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Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
915 920 925

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930 935 940

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945 950 955 960

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Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
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Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025 1030 1035 1040

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1105                1110                1115                1120

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                1125                1130                1135

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                1205                1210                1215

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            1220                1225                1230

CCA CCC AGC ACC TTC AAA GGG ACA CCT ACG GCA GAG AAC CCA GAG TAC      3744
Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245

CTG GGT CTG GAC GTG CCA GTG TGA ACC AGA AGG CCA AGT CCG CAG AAG      3792
Leu Gly Leu Asp Val Pro Val  *  Thr Arg Arg Pro Ser Pro Gln Lys
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Pro  *  Cys Val Leu Arg Glu Gln Gly Arg Pro Asp Phe Cys Trp His
1265                1270                1275                1280

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Gln Glu Val Gly Gly Pro Ser Asp His Phe Gln Gly Asn Leu Pro Cys
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GCT GGA AGG GGT CCA GCC TCG TTG GAA GAG GAA CAG CAC TGG GGA GTC 3984
Ala Gly Arg Gly Pro Ala Ser Leu Glu Glu Glu Gln His Trp Gly Val
1315 1320 1325

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Phe Val Asp Ser Glu Ala Leu Pro Asn Glu Thr Leu Gly Ser Ser Gly
1330 1335 1340

TGC CAC AGC CCA GCT TGG CCC TTT CCT TCC AGA TCC TGG GTA CTG AAA 4080
Cys His Ser Pro Ala Trp Pro Phe Pro Ser Arg Ser Trp Val Leu Lys
1345 1350 1355 1360

GCC TTA GGG AAG CTG GCC TGA GAG GGG AAG CGG CCC TAA GGG AGT GTC 4128
Ala Leu Gly Lys Leu Ala * Glu Gly Lys Arg Pro * Gly Ser Val
1365 1370 1375

TAA GAA CAA AAG CGA CCC ATT CAG AGA CTG TCC CTG AAA CCT AGT ACT 4176
* Glu Gln Lys Arg Pro Ile Gln Arg Leu Ser Leu Lys Pro Ser Thr
1380 1385 1390

GCC CCC CAT GAG GAA GGA ACA GCA ATG GTG TCA GTA TCC AGG CTT TGT 4224
Ala Pro His Glu Glu Gly Thr Ala Met Val Ser Val Ser Arg Leu Cys
1395 1400 1405

ACA GAG TGC TTT TCT GTT TAG TTT TTA CTT TTT TTG TTT TGT TTT TTT 4272
Thr Glu Cys Phe Ser Val * Phe Leu Leu Phe Leu Phe Cys Phe Phe
1410 1415 1420

AAA GAT GAA ATA AAG ACC CAG GGG GAG 4299
Lys Asp Glu Ile Lys Thr Gln Gly Glu
1425 1430

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1433 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
1 5 10 15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
20 25 30

Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
35 40 45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65 70 75 80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
115 120 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
130 135 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145 150 155 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
180 185 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
195 200 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
210 215 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225 230 235 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
245 250 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
260 265 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
275 280 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
290 295 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

```
Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
                325                 330                 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
                565                 570                 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605
```

```
Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
            660                 665                 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
        675                 680                 685

Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
    850                 855                 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865                 870                 875                 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895
```

```
Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945                 950                 955                 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
                965                 970                 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
            980                 985                 990

Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
        995                 1000                1005

Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
    1010                1015                1020

Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025                1030                1035                1040

Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
                1045                1050                1055

Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
            1060                1065                1070

Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
        1075                1080                1085

Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
    1090                1095                1100

Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105                1110                1115                1120

Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
                1125                1130                1135

Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
            1140                1145                1150

Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
        1155                1160                1165

Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
    1170                1175                1180
```

```
Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185                1190                1195                1200

Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
                1205                1210                1215

Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
            1220                1225                1230

Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245

Leu Gly Leu Asp Val Pro Val  *  Thr Arg Arg Pro Ser Pro Gln Lys
    1250                1255                1260

Pro  *  Cys Val Leu Arg Glu Gln Gly Arg Pro Asp Phe Cys Trp His
1265                1270                1275                1280

Gln Glu Val Gly Gly Pro Ser Asp His Phe Gln Gly Asn Leu Pro Cys
                1285                1290                1295

Gln Glu Pro Val Leu Arg Asn Leu Pro Ser Cys Leu Ser Ser Gln Met
            1300                1305                1310

Ala Gly Arg Gly Pro Ala Ser Leu Glu Glu Glu Gln His Trp Gly Val
        1315                1320                1325

Phe Val Asp Ser Glu Ala Leu Pro Asn Glu Thr Leu Gly Ser Ser Gly
    1330                1335                1340

Cys His Ser Pro Ala Trp Pro Phe Pro Ser Arg Ser Trp Val Leu Lys
1345                1350                1355                1360

Ala Leu Gly Lys Leu Ala  *  Glu Gly Lys Arg Pro  *  Gly Ser Val
                1365                1370                1375

 *  Glu Gln Lys Arg Pro Ile Gln Arg Leu Ser Leu Lys Pro Ser Thr
            1380                1385                1390

Ala Pro His Glu Glu Gly Thr Ala Met Val Ser Val Ser Arg Leu Cys
        1395                1400                1405

Thr Glu Cys Phe Ser Val  *  Phe Leu Leu Phe Leu Phe Cys Phe Phe
    1410                1415                1420

Lys Asp Glu Ile Lys Thr Gln Gly Glu
1425                1430
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 739 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..739
(D) OTHER INFORMATION: /note= "product = "520C9sFv/ amino acid info: 520C9sFv protein""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG 48
Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

ACA GTC AAG ATC TCC TGC AAG GCT TCT GGA TAT ACC TTC GCA AAC TAT 96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn Tyr
20 25 30

GGA ATG AAC TGG ATG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG ATG 144
Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

GGC TGG ATA AAC ACC TAC ACT GGA CAG TCA ACA TAT GCT GAT GAC TTC 192
Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp Phe
50 55 60

AAG GAA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC ACC ACT GCC CAT 240
Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala His
65 70 75 80

TTG CAG ATC AAC AAC CTC AGA AAT GAG GAC TCG GCC ACA TAT TTC TGT 288
Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe Cys
85 90 95

GCA AGA CGA TTT GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC AGT 336
Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Ser
100 105 110

GTC TCT GCA TCG ATA TCG AGC TCC TCC GGA TCT TCA TCT AGC GGT TCC 384
Val Ser Ala Ser Ile Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser
115 120 125

AGC TCG AGT GGA TCC GAT ATC CAG ATG ACC CAG TCT CCA TCC TCC TTA 432
Ser Ser Ser Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
130 135 140

TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG 480
Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln
145 150 155 160

GAC ATT GGT AAT AGC TTA ACC TGG CTT CAG CAG GAA CCA GAT GGA ACT 528
Asp Ile Gly Asn Ser Leu Thr Trp Leu Gln Gln Glu Pro Asp Gly Thr
165 170 175

```
ATT AAA CGC CTG ATC TAC GCC ACA TCC AGT TTA GAT TCT GGT GTC CCC     576
Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser Gly Val Pro
            180                 185                 190

AAA AGG TTC AGT GGC AGT CGG TCT GGG TCA GAT TAT TCT CTC ACC ATC     624
Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile
        195                 200                 205

AGT AGC CTT GAG TCT GAA GAT TTT GTA GTC TAT TAC TGT CTA CAA TAT     672
Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys Leu Gln Tyr
    210                 215                 220

GCT ATT TTT CCG TAC ACG TTC GGA GGG GGG ACC AAC CTG GAA ATA AAA     720
Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu Glu Ile Lys
225                 230                 235                 240

CGG GCT GAT TAA TCT GCA G                                           739
Arg Ala Asp  *  Ser Ala
                245
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn Tyr
            20                  25                  30

Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp Phe
    50                  55                  60

Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala His
65                  70                  75                  80

Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Ser
            100                 105                 110

Val Ser Ala Ser Ile Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser
        115                 120                 125
```

```
Ser Ser Ser Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
    130                 135                 140

Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln
145                 150                 155                 160

Asp Ile Gly Asn Ser Leu Thr Trp Leu Gln Gln Glu Pro Asp Gly Thr
                165                 170                 175

Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser Gly Val Pro
            180                 185                 190

Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile
        195                 200                 205

Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys Leu Gln Tyr
    210                 215                 220

Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu Glu Ile Lys
225                 230                 235                 240

Arg Ala Asp  *  Ser Ala
                245
```

(2) INFORMATION FOR SEQ ID NO:5: DELETED ACCORDING TO PRELIMINARY AMENDMENT

(2) INFORMATION FOR SEQ ID NO:6: DELETED ACCORDING TO PRELIMINARY AMENDMENT

(2) INFORMATION FOR SEQ IS NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 807 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..807
(D) OTHER INFORMATION: /note= "product = "Ricin-A chain gene/ amino acid info: Ricin-A chain protein""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG ATA TTC CCC AAA CAA TAC CCA ATT ATA AAC TTT ACC ACA GCG GGT      48
Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr Ala Gly
  1               5                  10                  15

GCC ACT GTG CAA AGC TAC ACA AAC TTT ATC AGA GCT GTT CGC GGT CGT      96
Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg Gly Arg
             20                  25                  30
```

```
TTA ACA ACT GGA GCT GAT GTG AGA CAT GAA ATA CCA GTG TTG CCA AAC      144
Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu Pro Asn
         35                  40                  45

AGA GTT GGT TTG CCT ATA AAC CAA CGG TTT ATT TTA GTT GAA CTC TCA      192
Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu Leu Ser
     50                  55                  60

AAT CAT GCA GAG CTT TCT GTT ACA TTA GCG CTG GAT GTC ACC AAT GCA      240
Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr Asn Ala
 65                  70                  75                  80

TAT GTG GTA GGC TAC CGT GCT GGA AAT AGC GCA TAT TTC TTT CAT CCT      288
Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe His Pro
                 85                  90                  95

GAC AAT CAG GAA GAT GCA GAA GCA ATC ACT CAT CTT TTC ACT GAT GTT      336
Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr Asp Val
            100                 105                 110

CAA AAT CGA TAT ACA TTC GCC TTT GGT GGT AAT TAT GAT AGA CTT GAA      384
Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg Leu Glu
        115                 120                 125

CAA CTT GCT GGT AAT CTG AGA GAA AAT ATC GAG TTG GGA AAT GGT CCA      432
Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn Gly Pro
    130                 135                 140

CTA GAG GAG GCT ATC TCA GCG CTT TAT TAT TAC AGT ACT GGT GGC ACT      480
Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly Gly Thr
145                 150                 155                 160

CAG CTT CCA ACT CTG GCT CGT TCC TTT ATA ATT TGC ATC CAA ATG ATT      528
Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln Met Ile
                165                 170                 175

TCA GAA GCA GCA AGA TTC CAA TAT ATT GAG GGA GAA ATG CGC ACG AGA      576
Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg Thr Arg
            180                 185                 190

ATT AGG TAC AAC CGG AGA TCT GCA CCA GAT CCT AGC GTA ATT ACA CTT      624
Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile Thr Leu
        195                 200                 205

GAG AAT AGT TGG GGG AGA CTT TCC ACT GCA ATT CAA GAG TCT AAC CAA      672
Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser Asn Gln
    210                 215                 220

GGA GCC TTT GCT AGT CCA ATT CAA CTG CAA AGA CGT AAT GGT TCC AAA      720
Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly Ser Lys
225                 230                 235                 240

TTC AGT GTG TAC GAT GTG AGT ATA TTA ATC CCT ATC ATA GCT CTC ATG      768
Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala Leu Met
                245                 250                 255
```

GTG TAT AGA TGC GCA CCT CCA CCA TCG TCA CAG TTT TAA 807
Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe
260 265

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 268 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr Ala Gly
1 5 10 15

Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg Gly Arg
20 25 30

Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu Pro Asn
35 40 45

Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu Leu Ser
50 55 60

Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr Asn Ala
65 70 75 80

Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe His Pro
85 90 95

Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr Asp Val
100 105 110

Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg Leu Glu
115 120 125

Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn Gly Pro
130 135 140

Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly Gly Thr
145 150 155 160

Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln Met Ile
165 170 175

Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg Thr Arg
180 185 190

Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile Thr Leu
195 200 205

```
Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser Asn Gln
    210                 215                 220

Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly Ser Lys
225                 230                 235                 240

Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala Leu Met
                245                 250                 255

Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe
            260                 265
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1605 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1605
(D) OTHER INFORMATION: /note= "product = "G-FIT""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
AAG CTT ATG ATA TTC CCC AAA CAA TAC CCA ATT ATA AAC TTT ACC ACA      48
Lys Leu Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr
  1               5                  10                  15

GCG GGT GCC ACT GTG CAA AGC TAC ACA AAC TTT ATC AGA GCT GTT CGC      96
Ala Gly Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg
             20                  25                  30

GGT CGT TTA ACA ACT GGA GCT GAT GTG AGA CAT GAA ATA CCA GTG TTG     144
Gly Arg Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu
         35                  40                  45

CCA AAC AGA GTT GGT TTG CCT ATA AAC CAA CGG TTT ATT TTA GTT GAA     192
Pro Asn Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu
     50                  55                  60

CTC TCA AAT CAT GCA GAG CTT TCT GTT ACA TTA GCG CTG GAT GTC ACC     240
Leu Ser Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr
 65                  70                  75                  80

AAT GCA TAT GTG GTA GGC TAC CGT GCT GGA AAT AGC GCA TAT TTC TTT     288
Asn Ala Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe
                 85                  90                  95
```

CAT CCT GAC AAT CAG GAA GAT GCA GAA GCA ATC ACT CAT CTT TTC ACT 336
His Pro Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr
100 105 110

GAT GTT CAA AAT CGA TAT ACA TTC GCC TTT GGT GGT AAT TAT GAT AGA 384
Asp Val Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg
115 120 125

CTT GAA CAA CTT GCT GGT AAT CTG AGA GAA AAT ATC GAG TTG GGA AAT 432
Leu Glu Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn
130 135 140

GGT CCA CTA GAG GAG GCT ATC TCA GCG CTT TAT TAT TAC AGT ACT GGT 480
Gly Pro Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly
145 150 155 160

GGC ACT CAG CTT CCA ACT CTG GCT CGT TCC TTT ATA ATT TGC ATC CAA 528
Gly Thr Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln
165 170 175

ATG ATT TCA GAA GCA GCA AGA TTC CAA TAT ATT GAG GGA GAA ATG CGC 576
Met Ile Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg
180 185 190

ACG AGA ATT AGG TAC AAC CGG AGA TCT GCA CCA GAT CCT AGC GTA ATT 624
Thr Arg Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile
195 200 205

ACA CTT GAG AAT AGT TGG GGG AGA CTT TCC ACT GCA ATT CAA GAG TCT 672
Thr Leu Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser
210 215 220

AAC CAA GGA GCC TTT GCT AGT CCA ATT CAA CTG CAA AGA CGT AAT GGT 720
Asn Gln Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly
225 230 235 240

TCC AAA TTC AGT GTG TAC GAT GTG AGT ATA TTA ATC CCT ATC ATA GCT 768
Ser Lys Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala
245 250 255

CTC ATG GTG TAT AGA TGC GCA CCT CCA CCA TCG TCA CAG TTT TCT CTT 816
Leu Met Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe Ser Leu
260 265 270

CTT ATA AGG CCA GTG GTA CCA AAT TTT AAT GCT GAT GTT TGT ATG GAT 864
Leu Ile Arg Pro Val Val Pro Asn Phe Asn Ala Asp Val Cys Met Asp
275 280 285

CCT GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA 912
Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly
290 295 300

GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGA TAT ACC TTC GCA AAC 960
Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn
305 310 315 320

TAT GGA ATG AAC TGG ATG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG 1008
Tyr Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp
325 330 335

ATG GGC TGG ATA AAC ACC TAC ACT GGA CAG TCA ACA TAT GCT GAT GAC 1056
Met Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp
340 345 350

TTC AAG GAA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC ACC ACT GCC 1104
Phe Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala
355 360 365

CAT TTG CAG ATC AAC AAC CTC AGA AAT GAG GAC TCG GCC ACA TAT TTC 1152
His Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe
370 375 380

TGT GCA AGA CGA TTT GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC 1200
Cys Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
385 390 395 400

AGT GTC TCT GCA TCG ATA TCG AGC TCT GGT GGC GGT GGC TCG GGC GGT 1248
Ser Val Ser Ala Ser Ile Ser Ser Ser Gly Gly Gly Gly Ser Gly Gly
405 410 415

GGT GGG TCG GGT GGC GGC GGA TCG GAT ATC CAG ATG ACC CAG TCT CCA 1296
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
420 425 430

TCC TCC TTA TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG 1344
Ser Ser Leu Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg
435 440 445

GCA AGT CAG GAC ATT GGT AAT AGC TTA ACC TGG CTT TCA CAG GAA CCA 1392
Ala Ser Gln Asp Ile Gly Asn Ser Leu Thr Trp Leu Ser Gln Glu Pro
450 455 460

GAT GGA ACT ATT AAA CGC CTG ATC TAC GCC ACA TCC AGT TTA GAT TCT 1440
Asp Gly Thr Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser
465 470 475 480

GGT GTC CCC AAA AGG TTC AGT GGC AGT CGG TCT GGG TCA GAT TAT TCT 1488
Gly Val Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser
485 490 495

CTC ACC ATC AGT AGC CTT GAG TCT GAA GAT TTT GTA GTC TAT TAC TGT 1536
Leu Thr Ile Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys
500 505 510

CTA CAA TAT GCT ATT TTT CCG TAC ACG TTC GGA GGG GGG ACC AAC CTG 1584
Leu Gln Tyr Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu
515 520 525

GAA ATA AAA CGG GCT GAT TAA 1605
Glu Ile Lys Arg Ala Asp
530 535

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 534 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Leu Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr
1 5 10 15

Ala Gly Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg
20 25 30

Gly Arg Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu
35 40 45

Pro Asn Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu
50 55 60

Leu Ser Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr
65 70 75 80

Asn Ala Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe
85 90 95

His Pro Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr
100 105 110

Asp Val Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg
115 120 125

Leu Glu Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn
130 135 140

Gly Pro Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly
145 150 155 160

Gly Thr Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln
165 170 175

Met Ile Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg
180 185 190

Thr Arg Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile
195 200 205

Thr Leu Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser
210 215 220

Asn Gln Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly
225 230 235 240

Ser Lys Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala
245 250 255

Leu Met Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe Ser Leu
260 265 270

Leu Ile Arg Pro Val Val Pro Asn Phe Asn Ala Asp Val Cys Met Asp
275 280 285

Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly
290 295 300

Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn
305 310 315 320

Tyr Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp
325 330 335

Met Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp
340 345 350

Phe Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala
355 360 365

His Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe
370 375 380

Cys Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
385 390 395 400

Ser Val Ser Ala Ser Ile Ser Ser Ser Gly Gly Gly Gly Ser Gly Gly
405 410 415

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
420 425 430

Ser Ser Leu Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg
435 440 445

Ala Ser Gln Asp Ile Gly Asn Ser Leu Thr Trp Leu Ser Gln Glu Pro
450 455 460

Asp Gly Thr Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser
465 470 475 480

Gly Val Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser
485 490 495

```
Leu Thr Ile Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys
            500                 505                 510

Leu Gln Tyr Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu
        515                 520                 525

Glu Ile Lys Arg Ala Asp
    530
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45
(D) OTHER INFORMATION: /note= "product = "new linker/ info: new linker""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TCG AGC TCC TCC GGA TCT TCA TCT AGC GGT TCC AGC TCG AGT GGA      45
Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45
(D) OTHER INFORMATION: /note= "product = "old linker/
protein info: old linker""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GGA GGA GGA GGA TCT GGA GGA GGA GGA TCT GGA GGA GGA GGA TCT          45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2001 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2001
(D) OTHER INFORMATION: /note= "product = "741sFv-PE40""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
GAT CCT GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT          48
Asp Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro
  1               5                  10                  15

GGA GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAT ACC TTC ACA          96
Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
             20                  25                  30
```

```
AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA AAG      144
Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys
        35                  40                  45

TGG ATG GGC TGG ATA AAC ACC AAC ACT GGA GAG CCA ACA TAT GCT GAA      192
Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu
    50                  55                  60

GAG TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC AGC ACT      240
Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr
65                  70                  75                  80

GCC TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT      288
Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr
                85                  90                  95

TTC TGT GGA AGG CAA TTT ATT ACC TAC GGC GGG TTT GCT AAC TGG GGC      336
Phe Cys Gly Arg Gln Phe Ile Thr Tyr Gly Gly Phe Ala Asn Trp Gly
            100                 105                 110

CAA GGG ACT CTG GTC ACT GTC TCT GCA TCG AGC TCC TCC GGA TCT TCA      384
Gln Gly Thr Leu Val Thr Val Ser Ala Ser Ser Ser Ser Gly Ser Ser
        115                 120                 125

TCT AGC GGT TCC AGC TCG AGC GAT ATC GTC ATG ACC CAG TCT CCT AAA      432
Ser Ser Gly Ser Ser Ser Ser Asp Ile Val Met Thr Gln Ser Pro Lys
    130                 135                 140

TTC ATG TCC ACG TCA GTG GGA GAC AGG GTC AGC ATC TCC TGC AAG GCC      480
Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Ser Cys Lys Ala
145                 150                 155                 160

AGT CAG GAT GTG AGT ACT GCT GTA GCC TGG TAT CAA CAA AAA CCA GGG      528
Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175

CAA TCT CCT AAA CTA CTG ATT TAC TGG ACA TCC ACC CGG CAC ACT GGA      576
Gln Ser Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg His Thr Gly
            180                 185                 190

GTC CCT GAT CCG TTC ACA GGC AGT GGA TCT GGG ACA GAT TAT ACT CTC      624
Val Pro Asp Pro Phe Thr Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu
        195                 200                 205

ACC ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA CTT CAT TAC TGT CAG      672
Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Leu His Tyr Cys Gln
    210                 215                 220

CAA CAT TAT AGA GTG GCC TAC ACG TTC GGA AGG GGG ACC AAG CTG GAG      720
Gln His Tyr Arg Val Ala Tyr Thr Phe Gly Arg Gly Thr Lys Leu Glu
225                 230                 235                 240

ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC      768
Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser
                245                 250                 255
```

```
AGT GAG CAG TTT GAG GGC GGC AGC CTG GCC GCG CTG AAC GCG CAC CAG      816
Ser Glu Gln Phe Glu Gly Gly Ser Leu Ala Ala Leu Asn Ala His Gln
            260                 265                 270

GCT TGC CAC CTG CCG CTG GAG ACT TTC ACC CGT CAT CGC CAG CCG CGC      864
Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        275                 280                 285

GGC TGG GAA CAA CTG GAG CAG TGC GGC TAT CCG GTG CAG CGG CTG GTC      912
Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    290                 295                 300

GCC CTC TAC CTG GCG GCG CGG CTG TCG TGG AAC CAG GTC GAC CAG GTG      960
Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
305                 310                 315                 320

ATC CGC AAC GCC CTG GCC AGC CCC GGC AGC GGC GGC GAC CTG GGC GAA     1008
Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                325                 330                 335

GCG ATC CGC GAG CAG CCG GAG CAG GCC CGT CTG GCC CTG ACC CTG GCC     1056
Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            340                 345                 350

GCC GCC GAG AGC GAG CGC TTC GTC CGG CAG GGC ACC GGC AAC GAC GAG     1104
Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        355                 360                 365

GCC GGC GCG GCC AAC GCC GAC GTG GTG AGC CTG ACC TGC CCG GTC GCC     1152
Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    370                 375                 380

GCC GGT GAA TGC GCG GGC CCG GCG GAC AGC GGC GAC GCC CTG CTG GAG     1200
Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
385                 390                 395                 400

CGC AAC TAT CCC ACT GGC GCG GAG TTC CTC GGC GAC GGC GGC GAC GTC     1248
Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                405                 410                 415

AGC TTC AGC AAC CGC GGC ACG CAG AAC TGG ACG GTG GAG CGG CTG CTC     1296
Ser Phe Ser Asn Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            420                 425                 430

CAG GCG CAC CGC CAA CTG GAG GAG CGC GGC TAT GTG TTC GTC GGC TAC     1344
Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        435                 440                 445

CAC GGC ACC TTC CTC GAA GCG GCG CAA AGC ATC GTC TTC GGC GGG GTG     1392
His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    450                 455                 460

CGC GCG CGC AGC CAG GAC CTC GAC GCG ATC TGG CGC GGT TTC TAT ATC     1440
Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
465                 470                 475                 480
```

```
GCC GGC GAT CCG GCG CTG GCC TAC GGC TAC GCC CAG GAC CAG GAA CCC     1488
Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
            485                 490                 495

GAC GCA CGC GGC CGG ATC CGC AAC GGT GCC CTG CTG CGG GTC TAT GTG     1536
Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
        500                 505                 510

CCG CGC TCG AGC CTG CCG GGC TTC TAC CGC ACC AGC CTG ACC CTG GCC     1584
Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        515                 520                 525

GCG CCG GAG GCG GCG GGC GAG GTC GAA CGG CTG ATC GGC CAT CCG CTG     1632
Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    530                 535                 540

CCG CTG CGC CTG GAC GCC ATC ACC GGC CCC GAG GAG GAA GGC GGG CGC     1680
Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
545                 550                 555                 560

CTG GAG ACC ATT CTC GGC TGG CCG CTG GCC GAG CGC ACC GTG GTG ATT     1728
Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                565                 570                 575

CCC TCG GCG ATC CCC ACC GAC CCG CGC AAC GTC GGC GGC GAC CTC GAC     1776
Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            580                 585                 590

CCG TCC AGC ATC CCC GAC AAG GAA CAG GCG ATC AGC GCC CTG CCG GAC     1824
Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        595                 600                 605

TAC GCC AGC CAG CCC GGC AAA CCG CCG CGC GAG GAC CTG AAG TAA CTG     1872
Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys  *  Leu
    610                 615                 620

CCG CGA CCG GCC GGC TCC CTT CGC AGG AGC CGG CCT TCT CGG GGC CTG     1920
Pro Arg Pro Ala Gly Ser Leu Arg Arg Ser Arg Pro Ser Arg Gly Leu
625                 630                 635                 640

GCC ATA CAT CAG GTT TTC CTG ATG CCA GCC CAA TCG AAT ATG AAT TGA     1968
Ala Ile His Gln Val Phe Leu Met Pro Ala Gln Ser Asn Met Asn  *
                645                 650                 655

TCC TCT AGA GTC GAC CTG CAG GCA TGC AAG CTT                         2001
Ser Ser Arg Val Asp Leu Gln Ala Cys Lys Leu
            660                 665
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 667 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Asp Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro
 1               5                  10                  15

Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
            20                  25                  30

Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys
        35                  40                  45

Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu
    50                  55                  60

Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr
65                  70                  75                  80

Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr
                85                  90                  95

Phe Cys Gly Arg Gln Phe Ile Thr Tyr Gly Gly Phe Ala Asn Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ala Ser Ser Ser Ser Gly Ser Ser
        115                 120                 125

Ser Ser Gly Ser Ser Ser Ser Asp Ile Val Met Thr Gln Ser Pro Lys
    130                 135                 140

Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Ser Cys Lys Ala
145                 150                 155                 160

Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175

Gln Ser Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg His Thr Gly
            180                 185                 190

Val Pro Asp Pro Phe Thr Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu
        195                 200                 205

Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Leu His Tyr Cys Gln
    210                 215                 220

Gln His Tyr Arg Val Ala Tyr Thr Phe Gly Arg Gly Thr Lys Leu Glu
225                 230                 235                 240

Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser
                245                 250                 255

Ser Glu Gln Phe Glu Gly Gly Ser Leu Ala Ala Leu Asn Ala His Gln
            260                 265                 270
```

Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
275 280 285

Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
290 295 300

Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
305 310 315 320

Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
325 330 335

Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
340 345 350

Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
355 360 365

Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
370 375 380

Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
385 390 395 400

Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
405 410 415

Ser Phe Ser Asn Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
420 425 430

Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
435 440 445

His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
450 455 460

Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
465 470 475 480

Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
485 490 495

Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
500 505 510

Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
515 520 525

Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
530 535 540

Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
545 550 555 560

Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
565 570 575

Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
580 585 590

Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
595 600 605

Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys * Leu
610 615 620

Pro Arg Pro Ala Gly Ser Leu Arg Arg Ser Arg Pro Ser Arg Gly Leu
625 630 635 640

Ala Ile His Gln Val Phe Leu Met Pro Ala Gln Ser Asn Met Asn *
645 650 655

Ser Ser Arg Val Asp Leu Gln Ala Cys Lys Leu
660 665

CLAIMS

1. A single-chain Fv (sFv) polypeptide defining a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen, said sFv comprising at least two polypeptide domains connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each of said polypeptide domains comprising a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), said CDRs conferring immunological binding to said c-erbB-2 or c-erbB-2-related tumor antigen.

2. The single-chain Fv polypeptide of claim 1 wherein said CDRs are substantially homologous with the CDRs of the c-erbB-2-binding immunoglobulin molecules selected from the group consisting of 520C9, 741F8, and 454C11 monoclonal antibodies.

3. The single-chain Fv polypeptide of claim 2 wherein the amino acid sequence of each of said sFv CDRs and each of said FRs are substantially homologous with the amino acid sequence of CDRs and FRs of the variable region of 520C9 antibody.

4. The single-chain Fv polypeptide of claim 1 wherein said polypeptide linker comprises the amino acid sequence as set forth in the Sequence Listing as amino acid residue numbers 118 through 133 in SEQ ID NO:4.

5. The single-chain Fv polypeptide of claim 1 wherein said polypeptide linker comprises an amino acid sequence selected from the group of sequences set forth as amino acid residues 116-135 in SEQ ID NO:6, or 122-135 in SEQ. ID NO:15 and the amino acid sequences set forth in SEQ ID NO: 12 and SEQ ID NO: 14.

6. The single-chain Fv polypeptide of claim 1 further comprising a remotely detectable moiety bound thereto to permit imaging of a cell bearing said c-erbB-2-related tumor antigen.

7. The single-chain Fv polypeptide of claim 6 wherein said remotely detectable moiety comprises a radioactive atom.

8. The single-chain Fv polypeptide of claim 1 further comprising, linked to the N or C terminus of said linked domains, a third polypeptide domain comprising an amino acid sequence defining CDRs interposed between FRs and defining a second immunologically active site.

9. The single-chain Fv polypeptide of claim 8, further comprising a fourth polypeptide domain, wherein said third and fourth polypeptide domains together comprise a second site which immunologically binds a c-erbB-2-related tumor antigen.

10. The single-chain Fv polypeptide of claim 1 or 7 further comprising a toxin linked to the N or C terminus of said linked domain.

11. The single-chain Fv polypeptide of claim 10 wherein said toxin comprises a toxic portion selected from the group: Pseudomonas exotoxin, ricin, ricin A chain, phytolaccin and diphtheria toxin.

12. The single-chain Fv polypeptide of claim 10 wherein said toxin comprises at least a portion of the ricin A chain.

13. A DNA sequence encoding the polypeptide chain of claim 1.

14. A method of producing a single chain polypeptide having specificity for a c-erbB-2-related tumor antigen, said method comprising the steps of:

(a) transfecting the DNA of claim 13 into a host cell to produce a transformant; and

(b) culturing said transformant to produce said single-chain polypeptide.

15. A method of imaging a tumor expressing a c-erbB-2-related antigen, said method comprising the steps of:

(a) providing an imaging agent comprising the polypeptide of claim 7;

(b) administering to a mammal harboring said tumor an amount of said imaging agent together with a physiologically-acceptable carrier sufficient to permit extracorporeal detection of said tumor after allowing said agent to bind to said tumor; and

(c) detecting the location of said remotely detectable moiety in said subject to obtain an image of said tumor.

16. A host cell transfected with a DNA of claim 13.

17. A method of inhibiting <u>in vivo</u> growth of a tumor expressing a c-erbB-2-related antigen, said method comprising:

administering to a patient harboring the tumor a tumor inhibiting amount of a therapeutic agent comprising a single-chain Fv of claim 1 and at least a first moiety peptide bonded thereto, said first moiety having the ability to limit the proliferation of a tumor cell.

18. The method of claim 17 wherein said first moiety comprises a cell toxin or a toxic fragment thereof.

19. The method of claim 17 wherein said first moiety comprises a radioisotope sufficiently radioactive to inhibit proliferation of said tumor cell.

20. A DNA sequence encoding the polypeptide chain of claim 10.

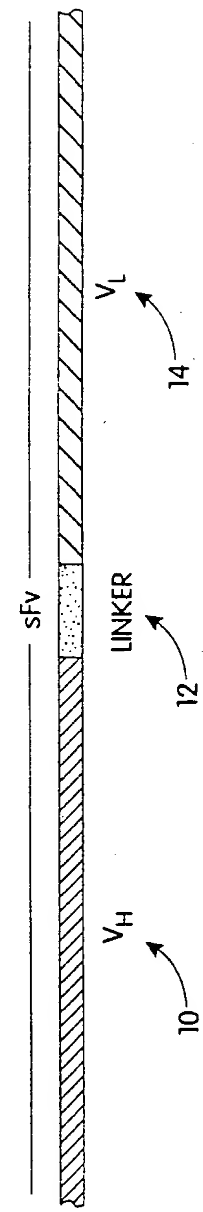


Fig. 1A

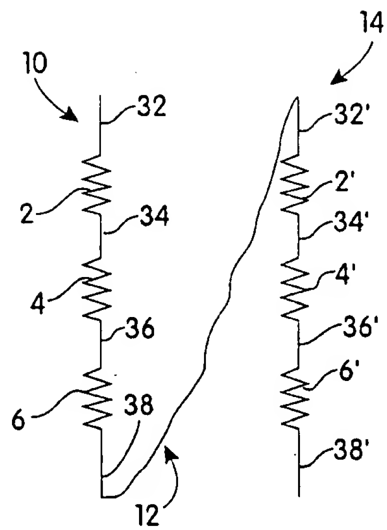


Fig. 1B

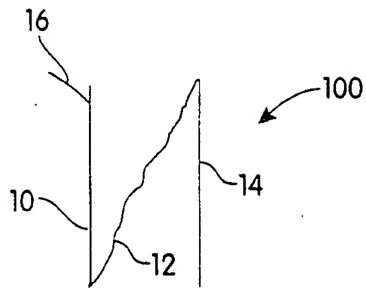


Fig. 2A

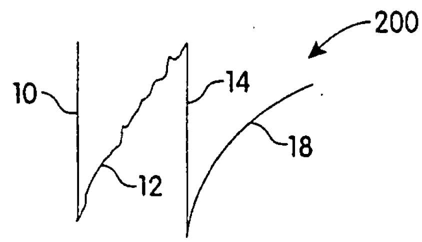


Fig. 2B

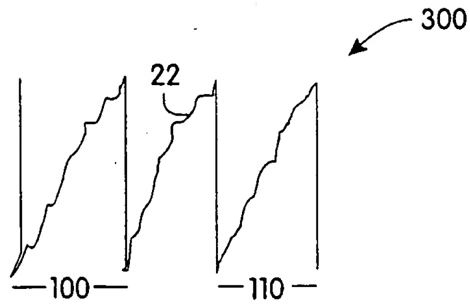


Fig. 2C

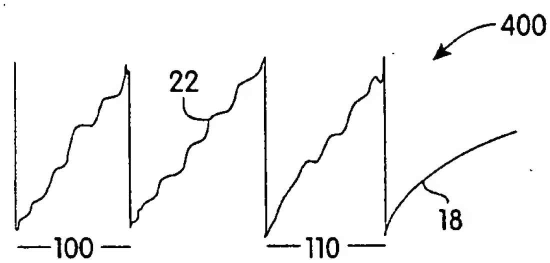


Fig. 2D

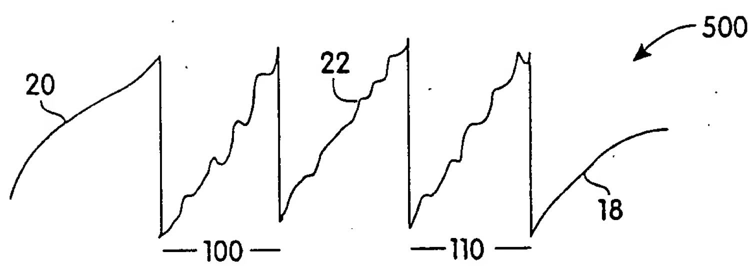
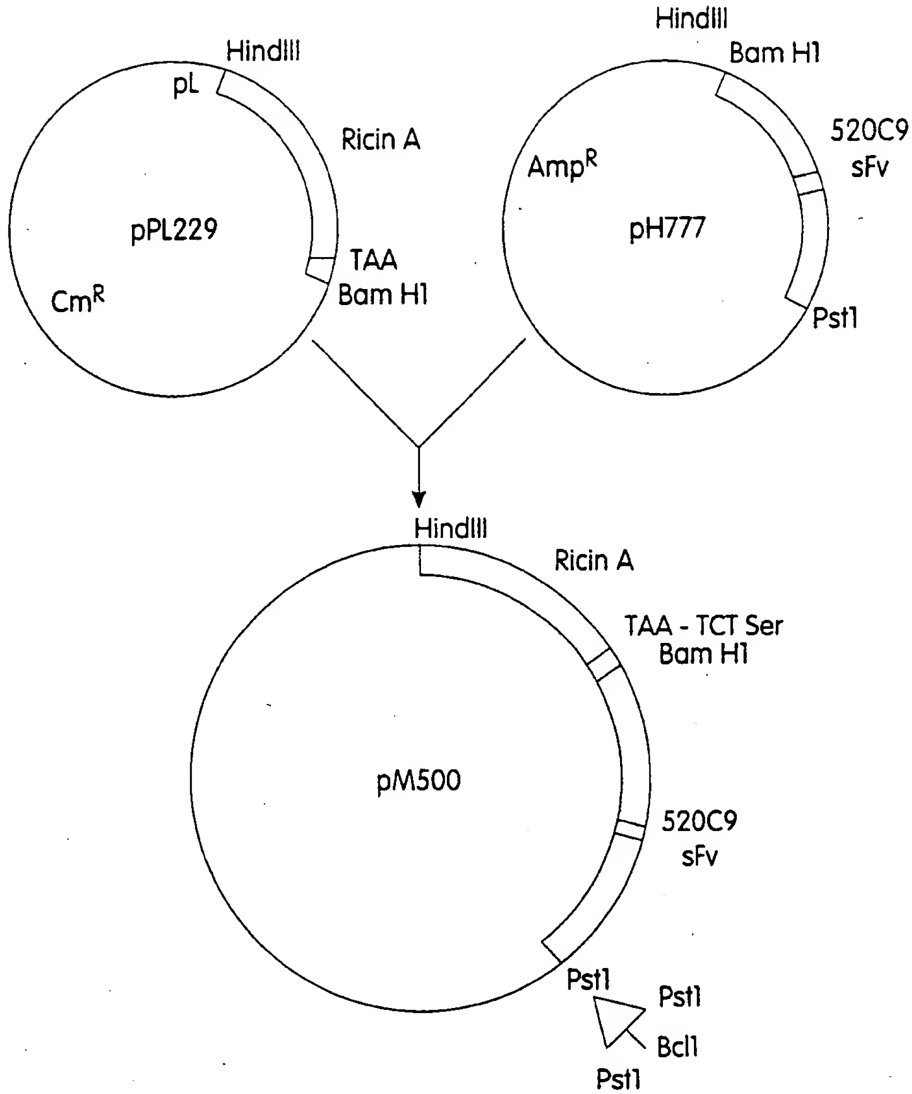


Fig. 2E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Ricin A
pPL229
TAA
Bam H1
CmR
HindIII
Bam H1
520C9
sFv
AmpR
pH777
Pst1
HindIII
Ricin A
TAA - TCT Ser
Bam H1
pM500
520C9
sFv
Pst1
Pst1
Bcl1
Pst1

Fig. 3

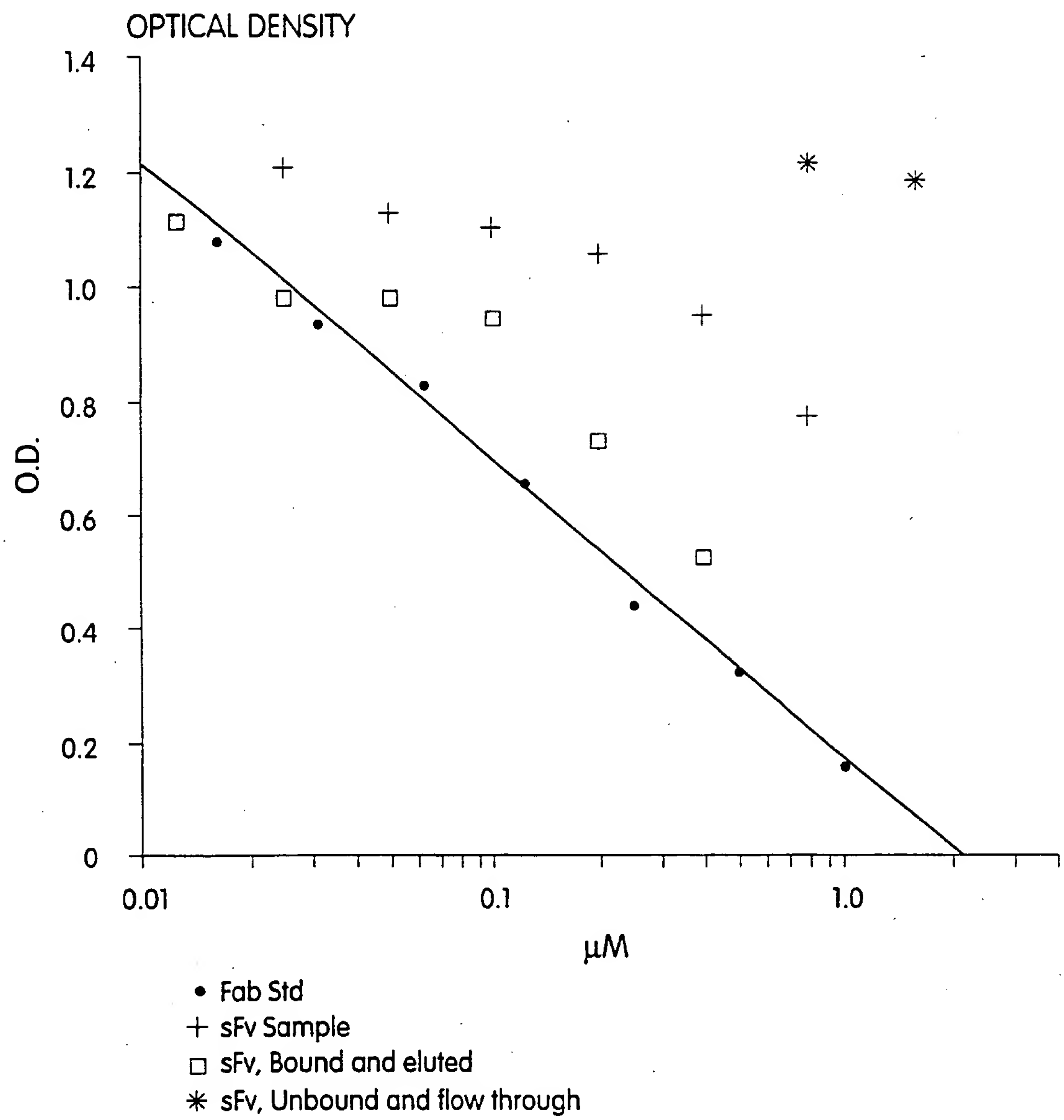
OPTICAL DENSITY
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
O.D.
0.01
0.1
1.0
μM
• Fab Std
+ sFv Sample
□ sFv, Bound and eluted
✳ sFv, Unbound and flow through

Fig. 4

170 175 180

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
185 190 195

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
200 205 210

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
215 220 225

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
230 235 240

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
245 250 255

Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala
260 265 270

Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
275 280 285

Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr Cys
290 295 300

Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
305 310 315

Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala Asn Gly
320 325 330

Pro His His Pro Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn
335 340 345

Gln Tyr Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
350 355 360

Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr
365 370 375

Ala His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
380 385 390

Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val
395 400 405

Ile Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro Thr
410 415 420

Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu
425 430 435

Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr
440 445 450

Arg Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr
455 460 465

Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
470 475 480

Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser Met Thr
485 490 495

Val Ser Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
500 505 510

Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu Lys Lys
515 520 525

Phe Asp His His Pro Gln Gln Phe Ser Ser Phe His His Asn Pro
530 535 540

Ala His Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
545 550 555

Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln
560 565 570

Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr
575 580 585

Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn
590 595 600

Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu
605 610 615

Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu
620 625 630

Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser
635 640 645

Arg Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln
650 655 660

Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
665 670 675

Xaa Asn Leu Asn Lys His Ile Asp Ser Pro Val Lys Leu Tyr Phe
680 685 690

Ile Xaa Xaa Ser Ile Pro Pro Xaa Ile Lys Gln Phe Ile Leu Phe
695 700 705

Xaa Gln Phe Cys Lys Xaa Lys Thr Gly Lys Lys Leu Leu Xaa Ile
710 715 720

Lys Tyr Met Tyr Val Lys Met Lys Lys Lys Lys Lys
725 730 732

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
1 5 10 15

Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser
20 25 30

Arg Tyr Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys
35 40 45

Thr Glu Asn Val Pro Met Lys Val Gln Asn Gln Glu Lys Ala Glu
50 55 60

Glu Leu Tyr Gln Lys Arg
65 66

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
1 5 10 15

Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser
20 25 30

Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys
35 40 45

Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu
50 55 60

Phe Met Glu Ala Glu Glu Leu Tyr Gln Lys Arg
65 70 71

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2010 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGCGCGAGC GCCTCAGCGC GGCCGCTCGC TCTCCCCCTC GAGGGACAAA 50

CTTTTCCCAA ACCCGATCCG AGCCCTTGGA CCAAACTCGC CTGCGCCGAG 100

AGCCGTCCGC GTAGAGCGCT CCGTCTCCGG CGAGATGTCC GAGCGCAAAG 150

AAGGCAGAGG CAAAGGGAAG GGCAAGAAGA AGGAGCGAGG CTCCGGCAAG 200

AAGCCGGAGT CCGCGGCGGG CAGCCAGAGC CCAGCCTTGC CTCCCCGATT 250

GAAAGAGATG AAAAGCCAGG AATCGGCTGC AGGTTCCAAA CTAGTCCTTC 300

GGTGTGAAAC CAGTTCTGAA TACTCCTCTC TCAGATTCAA GTGGTTCAAG 350

AATGGGAATG AATTGAATCG AAAAAACAAA CCACAAAATA TCAAGATACA 400

AAAAAAGCCA GGGAAGTCAG AACTTCGCAT TAACAAAGCA TCACTGGCTG 450

ATTCTGGAGA GTATATGTGC AAAGTGATCA GCAAATTAGG AAATGACAGT 500

GCCTCTGCCA ATATCACCAT CGTGGAATCA AACGAGATCA TCACTGGTAT 550

GCCAGCCTCA ACTGAAGGAG CATATGTGTC TTCAGAGTCT CCCATTAGAA 600

TATCAGTATC CACAGAAGGA GCAAATACTT CTTCATCTAC ATCTACATCC 650

ACCACTGGGA CAAGCCATCT TGTAAAATGT GCGGAGAAGG AGAAAACTTT 700

CTGTGTGAAT GGAGGGGAGT GCTTCATGGT GAAAGACCTT TCAAACCCCT 750

CGAGATACTT GTGCAAGTGC CAACCTGGAT TCACTGGAGC AAGATGTACT 800

GAGAATGTGC CCATGAAAGT CCAAAACCAA GAAAAGGCGG AGGAGCTGTA 850

CCAGAAGAGA GTGCTGACCA TAACCGGCAT CTGCATCGCC CTCCTTGTGG 900

TCGGCATCAT GTGTGTGGTG GCCTACTGCA AAACCAAGAA ACAGCGGAAA 950

AAGCTGCATG ACCGTCTTCG GCAGAGCCTT CGGTCTGAAC GAAACAATAT 1000

GATGAACATT GCCAATGGGC CTCACCATCC TAACCCACCC CCCGAGAATG 1050

TCCAGCTGGT GAATCAATAC GTATCTAAAA ACGTCATCTC CAGTGAGCAT 1100

ATTGTTGAGA GAGAAGCAGA GACATCCTTT TCCACCAGTC ACTATACTTC 1150

CACAGCCCAT CACTCCACTA CTGTCACCCA GACTCCTAGC CACAGCTGGA 1200

GCAACGGACA CACTGAAAGC ATCCTTTCCG AAAGCCACTC TGTAATCGTG 1250

ATGTCATCCG TAGAAAACAG TAGGCACAGC AGCCCAACTG GGGGCCCAAG 1300

AGGACGTCTT AATGGCACAG GAGGCCCTCG TGAATGTAAC AGCTTCCTCA 1350

GGCATGCCAG AGAAACCCCT GATTCCTACC GAGACTCTCC TCATAGTGAA 1400

AGGTATGTGT CAGCCATGAC CACCCCGGCT CGTATGTCAC CTGTAGATTT 1450

CCACACGCCA AGCTCCCCCA AATCGCCCCC TTCGGAAATG TCTCCACCCG 1500

TGTCCAGCAT GACGGTGTCC ATGCCTTCCA TGGCGGTCAG CCCCTTCATG 1550

GAAGAAGAGA GACCTCTACT TCTCGTGACA CCACCAAGGC TGCGGGAGAA 1600

GAAGTTTGAC CATCACCCTC AGCAGTTCAG CTCCTTCCAC CACAACCCCG 1650

CGCATGACAG TAACAGCCTC CCTGCTAGCC CCTTGAGGAT AGTGGAGGAT 1700

GAGGAGTATG AAACGACCCA AGAGTACGAG CCAGCCCAAG AGCCTGTTAA 1750

GAAACTCGCC AATAGCCGGC GGGCCAAAAG AACCAAGCCC AATGGCCACA 1800

TTGCTAACAG ATTGGAAGTG GACAGCAACA CAAGCTCCCA GAGCAGTAAC 1850

TCAGAGAGTG AAACAGAAGA TGAAAGAGTA GGTGAAGATA CGCCTTTCCT 1900

GGGCATACAG AACCCCCTGG CAGCCAGTCT TGAGGCAACA CCTGCCTTCC 1950

GCCTGGCTGA CAGCAGGACT AACCCAGCAG GCCGCTTCTC GACACAGGAA 2000

GAAATCCAGG 2010

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 669 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro Ser Arg Asp
1   5   10   15

Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly Pro Asn Ser Pro
                20   25   30

Ala Pro Arg Ala Val Arg Val Glu Arg Ser Val Ser Gly Glu Met
                35   40   45

Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys Lys
                50   55   60

Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln
                65   70   75

Ser Pro Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
80 85 90

Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser
95 100 105

Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu
110 115 120

Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys
125 130 135

Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala Asp
140 145 150

Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp
155 160 165

Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile
170 175 180

Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu
185 190 195

Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
200 205 210

Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys
215 220 225

Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
230 235 240

Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys
245 250 255

Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro
260 265 270

Met Lys Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln Lys
275 280 285

Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val
290 295 300

Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg
305 310 315

Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
320 325 330

Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro
335 340 345

Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
350 355 360

Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser
365 370 375

Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr
380 385 390

Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu

```
                395                     400                     405
Ser Ile Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val
                410                     415                     420
Glu Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg
                425                     430                     435
Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg
                440                     445                     450
His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser
                455                     460                     465
Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro
                470                     475                     480
Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu
                485                     490                     495
Met Ser Pro Pro Val Ser Ser Met Thr Val Ser Met Pro Ser Met
                500                     505                     510
Ala Val Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
                515                     520                     525
Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln
                530                     535                     540
Gln Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn Ser
                545                     550                     555
Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu
                560                     565                     570
Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu
                575                     580                     585
Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile
                590                     595                     600
Ala Asn Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser
                605                     610                     615
Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr
                620                     625                     630
Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala
                635                     640                     645
Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly
                650                     655                     660
Arg Phe Ser Thr Gln Glu Glu Ile Gln
                665                 669
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
 1               5                  10                  15

Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser
                20                  25                  30

Arg Tyr Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys
                35                  40                  45

Thr Glu Asn Val Pro Met Lys Val Gln Asn Gln Glu Lys Ala Glu
                50                  55                  60

Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile
                65                  70                  75

Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys
                80                  85                  90

Thr Lys Lys Gln Arg
                95
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu
 1               5                  10                  15

His Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala
                20                  25                  30

Cys Asn Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg
                35                  40                  45

Asp Leu Lys Trp Trp Glu Leu Arg His Ala Gly His Gly Gln Gln
                50                  55                  60

Gln Lys Val Ile Val Val Ala Val Cys Val Val Val Leu Val Met
                65                  70                  75

Leu Leu Leu Leu Ser Leu Trp Gly Ala His Tyr Tyr Arg Thr Gln
                80                  85                  90

Lys
 91
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Asn Asp Cys Pro Asp Ser His Thr Gln Phe Cys Phe His Gly Thr
 1               5                  10                  15
```

```
Cys Arg Phe Leu Val Gln Glu Asp Lys Pro Ala Cys Val Cys His
                20                  25                  30

Ser Gly Tyr Val Gly Ala Arg Cys Glu His Ala Asp Leu Leu Ala
                35                  40                  45

Val Val Ala Ala Ser Gln Lys Lys Gln Ala Ile Thr Ala Leu Val
                50                  55                  60

Val Val Ser Ile Val Ala Leu Ala Val Leu Ile Ile Thr Cys Val
                65                  70                  75

Leu Ile His Cys Cys Gln Val
                80      82
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Lys Lys Lys Asn Pro Cys Asn Ala Glu Phe Gln Asn Phe Cys Ile
 1              5                   10                  15

His Gly Glu Cys Lys Tyr Ile Glu His Leu Glu Ala Val Thr Cys
                20                  25                  30

Lys Cys Gln Gln Glu Tyr Phe Gly Glu Arg Cys Gly Glu Lys Ser
                35                  40                  45

Met Lys Thr His Ser Met Ile Asp Ser Ser Leu Ser Lys Ile Ala
                50                  55                  60

Leu Ala Ala Ile Ala Ala Phe Met Ser Ala Val Ile Leu Thr Ala
                65                  70                  75

Val Ala Val Ile Thr Val Gln Leu Arg Arg Gln Tyr
                80                  85      87
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Lys Lys Lys Asn Pro Cys Ala Ala Lys Phe Gln Asn Phe Cys Ile
 1              5                   10                  15

His Gly Glu Cys Arg Tyr Ile Glu Asn Leu Glu Val Val Thr Cys
                20                  25                  30

His Cys His Gln Asp Tyr Phe Gly Glu Arg Cys Gly Glu Lys Thr
                35                  40                  45

Met Lys Thr Gln Lys Lys Asp Asp Ser Asp Leu Ser Lys Ile Ala
                50                  55                  60
```

```
Leu Ala Ala Ile Ile Val Phe Val Ser Ala Val Ser Val Ala Ala
                65                  70                  75

Ile Gly Ile Ile Thr Ala Val Leu Leu Arg Lys Arg
                80                  85      87
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr Lys Asp Phe Cys Ile
 1              5                   10                  15

His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg Ala Pro Ser Cys
                20                  25                  30

Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His Gly Leu Ser
                35                  40                  45

Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr Thr Ile
                50                  55                  60

Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu Val
                65                  70                  75

Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg
                80                  85  86
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Arg Pro Asn Ala Arg Leu Pro Pro Gly Val Phe Tyr Cys
 1              5                   10          13
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCTCGCTCCT TCTTCTTGCC CTTCC 25

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 496 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
AA  AGA GCC GGC GAG GAG TTC CCC GAA ACT TGT TGG AAC 38
    Arg Ala Gly Glu Glu Phe Pro Glu Thr Cys Trp Asn
     1               5                  10

TCC GGG CTC GCG CGG AGG CCA GGA GCT GAG CGG CGG CGG 77
Ser Gly Leu Ala Arg Arg Pro Gly Ala Glu Arg Arg Arg
         15                  20                  25

CTG CCG GAC GAT GGG AGC GTG AGC AGG ACG GTG ATA ACC 116
Leu Pro Asp Asp Gly Ser Val Ser Arg Thr Val Ile Thr
                 30                  35

TCT CCC CGA TCG GGT TGC GAG GGC GCC GGG CAG AGG CCA 155
Ser Pro Arg Ser Gly Cys Glu Gly Ala Gly Gln Arg Pro
     40                  45                  50

GGA CGC GAG CCG CCA GCG GTG GGA CCC ATC GAC GAC TTC 194
Gly Arg Glu Pro Pro Ala Val Gly Pro Ile Asp Asp Phe
             55                  60

CCG GGG CGA CAG GAG CAG CCC CGA GAG CCA GGG CGA GCG 233
Pro Gly Arg Gln Glu Gln Pro Arg Glu Pro Gly Arg Ala
 65                  70                  75

CCC GTT CCA GGT GGC CGG ACC GCC CGC CGC GTC CGC GCC 272
Pro Val Pro Gly Gly Arg Thr Ala Arg Arg Val Arg Ala
         80                  85                  90

GCG CTC CCT GCA GGC AAC GGG AGA CGC CCC CGC GCA GCG 311
Ala Leu Pro Ala Gly Asn Gly Arg Arg Pro Arg Ala Ala
                 95                 100

CGA GCG CCT CAG CGC GGC CGC TCG CTC TCC CCC TCG AGG 350
Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro Ser Arg
    105                 110                 115

GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT GGA CCA 389
Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly Pro
            120                 125

AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG CGC TCC 428
Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu Arg Ser
130                 135                 140

GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC AGA GGC 467
Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly Arg Gly
        145                 150                 155

AAA GGG AAG GGC AAG AAG AAG GAG CGA GG 496
Lys Gly Lys Gly Lys Lys Lys Glu Arg
                160                 164
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2490 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
GTGGCTGCGG GGCAATTGAA AAAGAGCCGG CGAGGAGTTC CCCGAAACTT 50

GTTGGAACTC CGGGCTCGCG CGGAGGCCAG GAGCTGAGCG GCGGCGGCTG 100

CCGGACGATG GGAGCGTGAG CAGGACGGTG ATAACCTCTC CCCGATCGGG 150

TTGCGAGGGC GCCGGGCAGA GGCCAGGACG CGAGCCGCCA GCGGCGGGAC 200

CCATCGACGA CTTCCCGGGG CGACAGGAGC AGCCCCGAGA GCCAGGGCGA 250

GCGCCCGTTC CAGGTGGCCG GACCGCCCGC CGCGTCCGCG CCGCGCTCCC 300

TGCAGGCAAC GGGAGACGCC CCCGCGCAGC GCGAGCGCCT CAGCGCGGCC 350

GCTCGCTCTC CCCATCGAGG GACAAACTTT TCCCAAACCC GATCCGAGCC 400

CTTGGACCAA ACTCGCCTGC GCCGAGAGCC GTCCGCGTAG AGCGCTCCGT 450

CTCCGGCGAG  ATG TCC GAG CGC AAA GAA GGC AGA GGC AAA 490
            Met Ser Glu Arg Lys Glu Gly Arg Gly Lys
             1               5                  10

GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC AAG AAG 529
Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly Lys Lys
                 15                  20

CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA GCC TTG CCT 568
Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala Leu Pro
     25                  30                  35

CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG GCT GCA 607
Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala
             40                  45

GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT TCT GAA 646
Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
 50                  55                  60

TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG AAT GGG AAT 685
Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
         65                  70                  75
```

GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT ATC AAG ATA 724
Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile
80 85

CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC ATT AAC AAA 763
Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys
90 95 100

GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC AAA GTG 802
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
105 110

ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC AAT ATC 841
Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile
115 120 125

ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT GGT ATG CCA 880
Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met Pro
130 135 140

GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA GAG TCT CCC 919
Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu Ser Pro
145 150

ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA AAT ACT TCT 958
Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
155 160 165

TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA AGC CAT CTT 997
Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu
170 175

GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT 1036
Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
180 185 190

GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA AAC CCC 1075
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro
195 200 205

TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT 1114
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly
210 215

GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAG 1153
Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys
220 225 230

GCG GAG GAG CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC 1192
Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr
235 240

GGC ATC TGC ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT 1231
Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys
245 250 255

GTG GTG GCC TAC TGC AAA ACC AAG AAA CAG CGG AAA AAG 1270
Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
260 265 270

CTG CAT GAC CGT CTT CGG CAG AGC CTT CGG TCT GAA CGA 1309
Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
275 280

```
AAC AAT ATG ATG AAC ATT GCC AAT GGG CCT CAC CAT CCT 1348
Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro
    285                     290                 295

AAC CCA CCC CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC 1387
Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr
            300                 305

GTA TCT AAA AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG 1426
Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
310                 315                 320

AGA GAA GCA GAG ACA TCC TTT TCC ACC AGT CAC TAT ACT 1465
Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr
        325                 330                 335

TCC ACA GCC CAT CAC TCC ACT ACT GTC ACC CAG ACT CCT 1504
Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
            340                 345

AGC CAC AGC TGG AGC AAC GGA CAC ACT GAA AGC ATC CTT 1543
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Leu
    350                 355                 360

TCC GAA AGC CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA 1582
Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
            365                 370

AAC AGT AGG CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA 1621
Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly
375                 380                 385

CGT CTT AAT GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC 1660
Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser
        390                 395                 400

TTC CTC AGG CAT GCC AGA GAA ACC CCT GAT TCC TAC CGA 1699
Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg
            405                 410

GAC TCT CCT CAT AGT GAA AGG TAT GTG TCA GCC ATG ACC 1738
Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr
    415                 420                 425

ACC CCG GCT CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA 1777
Thr Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro
            430                 435

AGC TCC CCC AAA TCG CCC CCT TCG GAA ATG TCT CCA CCC 1816
Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro Pro
440                 445                 450

GTG TCC AGC ATG ACG GTG TCC AAG CCT TCC ATG GCG GTC 1855
Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
        455                 460                 465

AGC CCC TTC ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG 1894
Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
            470                 475

ACA CCA CCA AGG CTG CGG GAG AAG AAG TTT GAC CAT CAC 1933
Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His
    480                 485                 490
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CCT CAG CAG TTC AGC TCC TTC CAC CAC AAC CCC GCG CAT 1972
Pro Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His
            495                 500

GAC AGT AAC AGC CTC CCT GCT AGC CCC TTG AGG ATA GTG 2011
Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
505                 510                 515

GAG GAT GAG GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA 2050
Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro
        520                 525                 530

GCC CAA GAG CCT GTT AAG AAA CTC GCC AAT AGC CGG CGG 2089
Ala Gln Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg
                535                 540

GCC AAA AGA ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA 2128
Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn Arg
    545                 550                 555

TTG GAA GTG GAC AGC AAC ACA AGC TCC CAG AGC AGT AAC 2167
Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn
            560                 565

TCA GAG AGT GAA ACA GAA GAT GAA AGA GTA GGT GAA GAT 2206
Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp
570                 575                 580

ACG CCT TTC CTG GGC ATA CAG AAC CCC CTG GCA GCC AGT 2245
Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser
        585                 590                 595

CTT GAG GCA ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG 2284
Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg
                600                 605

ACT AAC CCA GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC 2323
Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile
    610                 615                 620

CAG GCC AGG CTG TCT AGT GTA ATT GCT AAC CAA GAC CCT 2362
Gln Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro
            625                 630

ATT GCT GTA TAAAACCTA AATAAACACA TAGATTCACC TGTAAAACTT 2410
Ile Ala Val
635     637

TATTTTATAT AATAAAGTAT TCCACCTTAA ATTAAACAAT TTATTTTATT 2460

TTAGCAGTTC TGCAAATAAA AAAAAAAAAA 2490
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1715 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
GCGCCTGCCT CCAACCTGCG GGCGGGAGGT GGGTGGCTGC GGGGCAATTG 50

AAAAAGAGCC GGCGAGGAGT TCCCCGAAAC TTGTTGGAAC TCCGGGCTCG 100

CGCGGAGGCC AGGAGCTGAG CGGCGGCGGC TGCCGGACGA TGGGAGCGTG 150

AGCAGGACGG TGATAACCTC TCCCCGATCG GGTTGCGAGG GCGCCGGGCA 200

GAGGCCAGGA CGCGAGCCGC CAGCGGCGGG ACCCATCGAC GACTTCCCGG 250

GGCGACAGGA GCAGCCCCGA GAGCCAGGGC GAGCGCCCGT TCCAGG[illegible]GC 300

CGGACCGCCC GCCGCGTCCG CGCCGCGCTC CCTGCAGGCA ACGGGAGACG 350

CCCCCGCGCA GCGCGAGCGC CTCAGCGCGG CCGCTCGCTC TCCCCATCGA 400

GGGACAAACT TTTCCCAAAC CCGATCCGAG CCCTTGGACC AAACTCGCCT 450

GCGCCGAGAG CCGTCCGCGT AGAGCGCTCC GTCTCCGGCG AG   ATG 495
                                                 Met
                                                   1

TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 534
Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
            5                   10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 573
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 612
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
         30                  35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 651
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                 45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 690
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 729
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
             70                  75

AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 768
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                  85                  90
```

```
GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 807
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
         95                 100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 846
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
            110                 115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 885
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 924
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
        135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 963
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1002
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1041
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
            175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1080
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1119
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
        200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1158
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC 1197
Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro
        225                 230                 235

TTT CTG TCT CTG CCT GAA TAGGA GCATGCTCAG TTGGTGCTGC 1240
Phe Leu Ser Leu Pro Glu
                240 241

TTTCTTGTTG CTGCATCTCC CCTCAGATTC CACCTAGAGC TAGATGTGTC 1290

TTACCAGATC TAATATTGAC TGCCTCTGCC TGTCGCATGA GAACATTAAC 1340

AAAAGCAATT GTATTACTTC CTCTGTTCGC GACTAGTTGG CTCTGAGATA 1390

CTAATAGGTG TGTGAGGCTC CGGATGTTTC TGGAATTGAT ATTGAATGAT 1440

GTGATACAAA TTGATAGTCA ATATCAAGCA GTGAAATATG ATAATAAAGG 1490

CATTTCAAAG TCTCACTTTT ATTGATAAAA TAAAAATCAT TCTACTGAAC 1540
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AGTCCATCTT CTTTATACAA TGACCACATC CTGAAAAGGG TGTTGCTAAG 1590

CTGTAACCGA TATGCACTTG AAATGATGGT AAGTTAATTT TGATTCAGAA 1640

TGTGTTATTT GTCACAAATA AACATAATAA AAGGAGTTCA GATGTTTTTC 1690

TTCATTAACC AAAAAAAAAA AAAAA 1715

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2431 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: N.A.
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GAGGCGCCTG CCTCCAACCT GCGGGCGGGA GGTGGGTGGC TGCGGGGCAA 50

TTGAAAAAGA GCCGGCGAGG AGTTCCCCGA AACTTGTTGG AACTCCGGGC 100

TCGCGCGGAG GCCAGGAGCT GAGCGGCGGC GGCTGCCGGA CGATGGGAGC 150

GTGAGCAGGA CGGTGATAAC CTCTCCCCGA TCGGGTTGCG AGGGCGCCGG 200

GCAGAGGCCA GGACGCGAGC CGCCAGCGGC GGGACCCATC GACGACTTCC 250

CGGGGCGACA GGAGCAGCCC CGAGAGCCAG GGCGAGCGCC CGTTCCAGGT 300

GGCCGGACCG CCCGCCGCGT CCGCGCCGCG CTCCCTGCAG GCAACGGGAG 350

ACGCCCCCGC GCAGCGCGAG CGCCTCAGCG CGGCCGCTCG CTCTCCCCAT 400

CGAGGGACAA ACTTTTCCCA AACCCGATCC GAGCCCTTGG ACCAAACTCG 450

CCTGCGCCGA GAGCCGTCCG CGTAGAGCGC TCCGTCTCCG GCGAG AT 497
Met
1

```
G TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 537
  Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
              5                   10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 576
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25
```

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GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 615
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
         30                 35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 654
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                 45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 693
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 732
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
             70                  75

AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 771
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
80                  85                  90

GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 810
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
         95                 100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 849
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                110                 115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 888
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 927
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
        135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 966
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1005
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1044
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1083
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1122
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1161
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AAG GCG GAG GAG CTG 1200
Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu
        225                 230                 235
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TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC TGC ATC 1239
Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile
                240                 245

GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC 1278
Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr
    250                 255                 260

TGC AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT 1317
Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg
            265                 270

CTT CGG CAG AGC CTT CGG TCT GAA CGA AAC AAT ATG ATG 1356
Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met Met
275                 280                 285

AAC ATT GCC AAT GGG CCT CAC CAT CCT AAC CCA CCC CCC 1395
Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
        290                 295                 300

GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA TCT AAA AAC 1434
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
                305                 310

GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG 1473
Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
    315                 320                 325

ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT 1512
Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His
            330                 335

CAC TCC ACT ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG 1551
His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
340                 345                 350

AGC AAC GGA CAC ACT GAA AGC ATC CTT TCC GAA AGC CAC 1590
Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His
        355                 360                 365

TCT GTA ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC 1629
Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His
                370                 375

AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT GGC 1668
Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly
    380                 385                 390

ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT 1707
Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His
            395                 400

GCC AGA GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT 1746
Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His
405                 410                 415

AGT GAA AGG TAAAA CCGAAGGCAA AGCTACTGCA GAGGAGAAAC 1790
Ser Glu Arg
        420

TCAGTCAGAG AATCCCTGTG AGCACCTGCG GTCTCACCTC AGGAAATCTA 1840
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CTCTAATCAG AATAAGGGGC GGCAGTTACC TGTTCTAGGA GTGCTCCTAG 1890

TTGATGAAGT CATCTCTTTG TTTGACGGAA CTTATTTCTT CTGAGCTTCT 1940

CTCGTCGTCC CAGTGACTGA CAGGCAACAG ACTCTTAAAG AGCTGGGATG 1990

CTTTGATGCG GAAGGTGCAG CACATGGAGT TTCCAGCTCT GGCCATGGGC 2040

TCAGACCCAC TCGGGGTCTC AGTGTCCTCA GTTGTAACAT TAGAGAGATG 2090

GCATCAATGC TTGATAAGGA CCCTTCTATA ATTCCAATTG CCAGTTATCC 2140

AAACTCTGAT TCGGTGGTCG AGCTGGCCTC GTGTTCTTAT CTGCTAACCC 2190

TGTCTTACCT TCCAGCCTCA GTTAAGTCAA ATCAAGGGCT ATGTCATTGC 2240

TGAATGTCAT GGGGGGCAAC TGCTTGCCCT CCACCCTATA GTATCTATTT 2290

TATGAAATTC CAAGAAGGGA TGAATAAATA AATCTCTTGG ATGCTGCGTC 2340

TGGCAGTCTT CACGGGTGGT TTTCAAAGCA GAAAAAAAAA AAAAAAAAAA 2390

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 2431

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
1 5 10 15

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
20 25 30

Gln Ser Pro Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln
35 40 45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
50 55 60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
65 70 75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
80 85 90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
95 100 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
110 115 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
125 130 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
140 145 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
155 160 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
170 175 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
185 190 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
200 205 210

Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val
215 220 225

Pro Met Lys Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln
230 235 240

Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val
245 250 255

Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln
260 265 270

Arg Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu
275 280 285

Arg Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn
290 295 300

Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys
305 310 315

Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr
320 325 330

Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
335 340 345

Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr
350 355 360

Glu Ser Ile Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser
365 370 375

Val Glu Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly
380 385 390

Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu
395 400 405

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Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His
                410                 415                 420

Ser Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser
                425                 430                 435

Pro Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser
                440                 445                 450

Glu Met Ser Pro Pro Val Ser Ser Met Thr Val Ser Met Pro Ser
                455                 460                 465

Met Ala Val Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu
                470                 475                 480

Val Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His Pro
                485                 490                 495

Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn
                500                 505                 510

Ser Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr
                515                 520                 525

Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys
                530                 535                 540

Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His
                545                 550                 555

Ile Ala Asn Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser
                560                 565                 570

Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp
                575                 580                 585

Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu
                590                 595                 600

Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala
                605                 610                 615

Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln
                620                 625
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 645 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
1               5                   10                  15

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
                20                  25                  30

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
                35                  40                  45
```

```
Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
                50                  55                  60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
                65                  70                  75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
                80                  85                  90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
                95                  100                 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
                110                 115                 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
                125                 130                 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
                140                 145                 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
                155                 160                 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
                170                 175                 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
                185                 190                 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
                200                 205                 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
                215                 220                 225

Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala
                230                 235                 240

Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
                245                 250                 255

Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr Cys
                260                 265                 270

Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
                275                 280                 285

Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala Asn Gly
                290                 295                 300

Pro His His Pro Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn
                305                 310                 315

Gln Tyr Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
                320                 325                 330

Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr
                335                 340                 345

Ala His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
                350                 355                 360
```

Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val
365 370 375

Ile Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro Thr
380 385 390

Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu
395 400 405

Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr
410 415 420

Arg Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr
425 430 435

Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
440 445 450

Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser Met Thr
455 460 465

Val Ser Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
470 475 480

Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu Lys Lys
485 490 495

Phe Asp His His Pro Gln Gln Phe Ser Ser Phe His His Asn Pro
500 505 510

Ala His Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
515 520 525

Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln
530 535 540

Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr
545 550 555

Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn
560 565 570

Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu
575 580 585

Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu
590 595 600

Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser
605 610 615

Arg Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln
620 625 630

Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
635 640 645

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 637 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
 1               5                  10                  15

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
                20                  25                  30

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
                35                  40                  45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
                50                  55                  60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
                65                  70                  75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
                80                  85                  90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
                95                 100                 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
               110                 115                 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
               125                 130                 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
               140                 145                 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
               155                 160                 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
               170                 175                 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
               185                 190                 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
               200                 205                 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
               215                 220                 225

Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val
               230                 235                 240

Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile
               245                 250                 255

Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
               260                 265                 270

Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn
               275                 280                 285

Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
               290                 295                 300
```

Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile
305 310 315

Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe Ser
320 325 330

Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr
335 340 345

Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile
350 355 360

Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu Asn
365 370 375

Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
380 385 390

Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala
395 400 405

Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
410 415 420

Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp
425 430 435

Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser
440 445 450

Pro Pro Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
455 460 465

Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro
470 475 480

Pro Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln Gln Phe
485 490 495

Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn Ser Leu Pro
500 505 510

Ala Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr
515 520 525

Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu Ala Asn
530 535 540

Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn
545 550 555

Arg Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn Ser
560 565 570

Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe
575 580 585

Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro
590 595 600

Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe
605 610 615

```
Ser Thr Gln Glu Glu Ile Gln Ala Arg Leu Ser Ser Val Ile Ala
                620                 625                 630

Asn Gln Asp Pro Ile Ala Val
                635     637
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
 1               5                   10                  15

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
                20                  25                  30

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
                35                  40                  45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
                50                  55                  60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
                65                  70                  75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
                80                  85                  90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
                95                  100                 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
                110                 115                 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
                125                 130                 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
                140                 145                 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
                155                 160                 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
                170                 175                 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
                185                 190                 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
                200                 205                 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
                215                 220                 225

Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val
                230                 235                 240
```

Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile
245 250 255

Met Cys Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
260 265 270

Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn
275 280 285

Met Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
290 295 300

Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile
305 310 315

Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe Ser
320 325 330

Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr
335 340 345

Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile
350 355 360

Leu Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu Asn
365 370 375

Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
380 385 390

Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala
395 400 405

Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
410 415 420

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys
1 5 10 15

Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser
20 25 30

Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln
35 40 45

Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
50 55 60

Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
65 70 75

Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys
80 85 90

Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
95 100 105

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn
110 115 120

Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile
125 130 135

Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
140 145 150

Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr
155 160 165

Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
170 175 180

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
185 190 195

Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys
200 205 210

Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val
215 220 225

Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro
230 235 240

Glu
241

WE CLAIM:

1. A composition comprising isolated heregulin polypeptide.

2. The composition of claim 1 wherein the heregulin is antigenically active.

3. The composition of claim 1 wherein the heregulin is biologically active.

4. The composition of claim 3 wherein the heregulin is HRG-GFD.

5. The composition of claim 1 wherein the heregulin is heregulin -α, −β1, −β2, or −β3.

6. The composition of claim 3 wherein the heregulin is human heregulin-α−GFD.

7. The composition of claim 3 wherein the heregulin is human heregulin-β1−GFD, heregulin-β2−GFD or heregulin-β3−GFD .

8. The composition of claim 1 further comprising pharmaceutically acceptable carrier.

9. The composition of claim 8 wherein the heregulin is a heregulin GFD.

10. The composition of claim 9 further comprising an immune adjuvant.

11. The composition of claim 10 wherein the heregulin GFD comprises an immunogenic, non-heregulin polypeptide.

12. The composition of claim 1 wherein the heregulin is NTD-GFD.

13. The composition of claim 1 wherein the heregulin is NTD-GFD-transmembrane polypeptide.

14. The composition of claim 1 wherein the heregulin is HRG-GFD.

15. The composition of claim 1 wherein the heregulin comprises a cytoplasmic domain.

16. The composition of claim 1 wherein the heregulin is NTD-GFD and it has an amino acid sequence which is at least 85% homologous with the native heregulin-α, −β1. −β2, −β3 NTD-GFD sequence.

17. The composition of claim 1 wherein the heregulin polypeptide comprises an enzyme.

18. The composition of claim 16 wherein the heregulin is HRG-α.

19. The composition of claim 18 wherein the heregulin-α has an amino acid substituted, deleted or inserted adjacent to any one of residues 1-23, 107-108,121-123, 128-130 and 163-247 (Fig. 15).

20. The composition of claim 16 wherein the heregulin is HRG-β_1.

21. The composition of claim 20 wherein the heregulin β_1 has an amino acid substituted, deleted or inserted adjacent to residues 1-23, 107-108, 121-123, 128-130 and 163-252 (Fig. 15).

22. The composition of claim 16 wherein the heregulin is HRG-β_2.

23. The composition of claim 22 wherein the heregulin β_2 has an amino acid substituted, deleted or inserted adjacent to any one of residues 1-23, 107-108, 121-123, 128-130 and 163-244 (Fig. 15).

24. The composition of claim 16 wherein the heregulin is HRG-β_3.

25. The composition of claim 24 wherein the heregulin β_3 has an amino acid substituted, deleted or inserted adjacent to any one of residues 1-23, 107-108, 121-123, 128-130 and 163-241 (Fig. 15).

26. An isolated antibody that is capable of binding a heregulin polypeptide.

27. The isolated antibody of claim 26 that is capable of binding specifically to a heregulin-α, heregulin-β1, heregulin-β_2, or heregulin-β3.

28. Isolated heregulin encoding nucleic acid.

29. The nucleic acid of claim 28 which encodes heregulin-α, heregulin-β1, heregulin-β2, or heregulin-β3 polypeptide.

30. The nucleic acid of claim 28 that encodes a heregulin-GFD.

31. An expression vector comprising the nucleic acid of claim 28.

32. The expression vector of claim 31 wherein the nucleic acid encodes a heregulin-GFD.

33. A host cell transformed with a vector of claim 31.

34. A method comprising culturing the host cell of claim 33 to express the heregulin and recovering the heregulin from the host cell.

35. The method of claim 34 wherein the heregulin is heregulin-α, heregulin-β1, heregulin β2, or heregulin-β3.

36. The method of claim 34 wherein the heregulin is heregulin-NTD-GFD.

37. The method of claim 34 wherein the heregulin is heregulin-GFD.

38. A method of determining the presence of a heregulin nucleic acid, comprising contacting the nucleic acid of claim 28 with a test sample nucleic acid and determining whether hybridization has occurred.

39. A method of amplifying a nucleic acid test sample comprising priming a nucleic acid polymerase chain reaction with the nucleic acid of claim 28.

40. A method for purifying a heregulin comprising adsorbing heregulin from a contaminated solution thereof onto heparin Sepharose or a cation exchange resin.

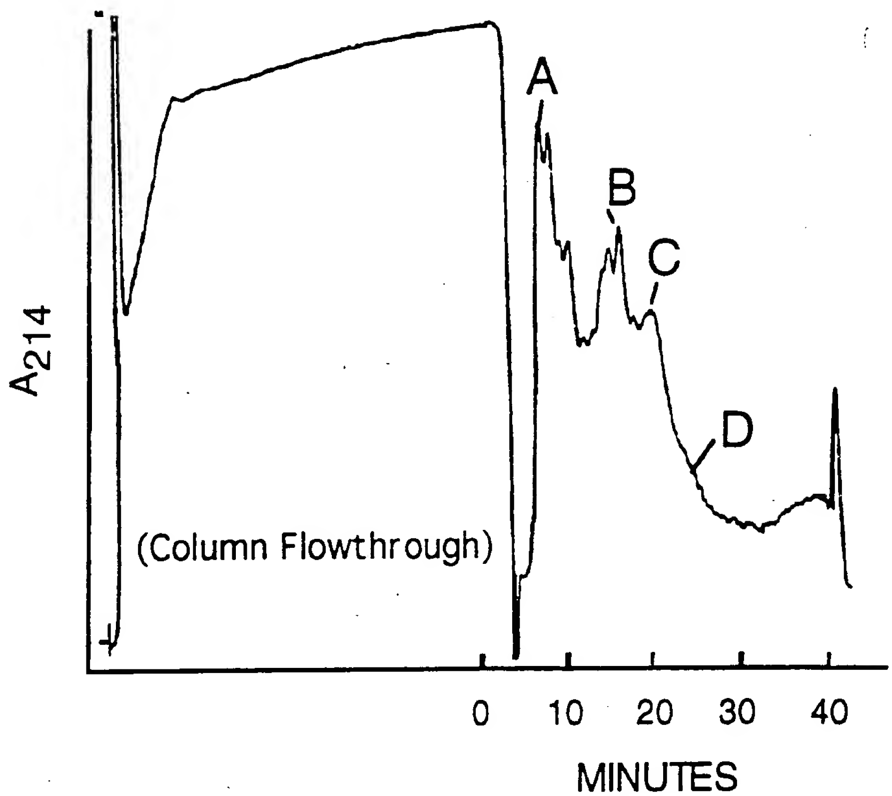

FIG. 1

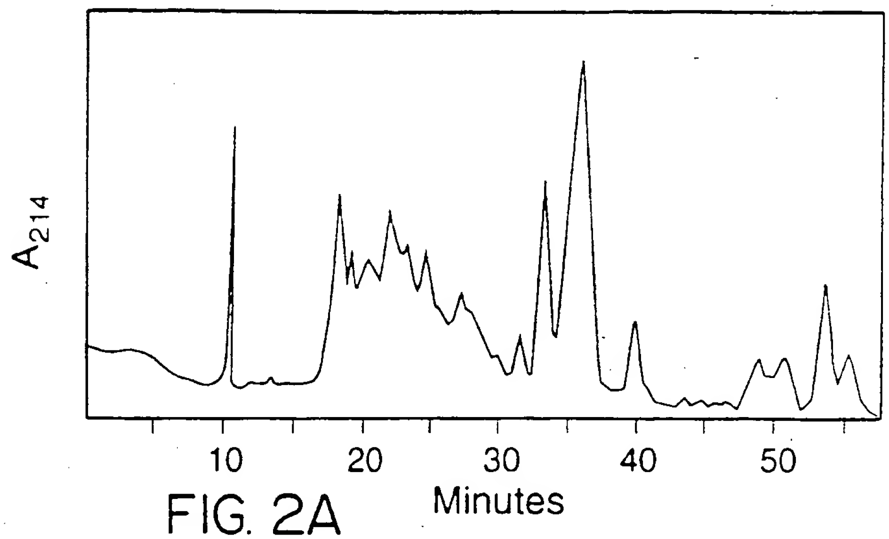

FIG. 2A

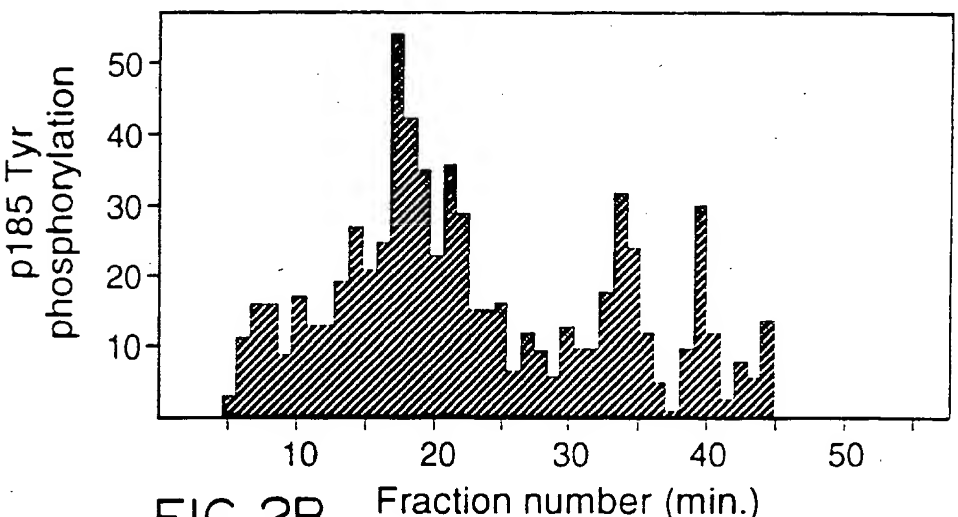

FIG. 2B

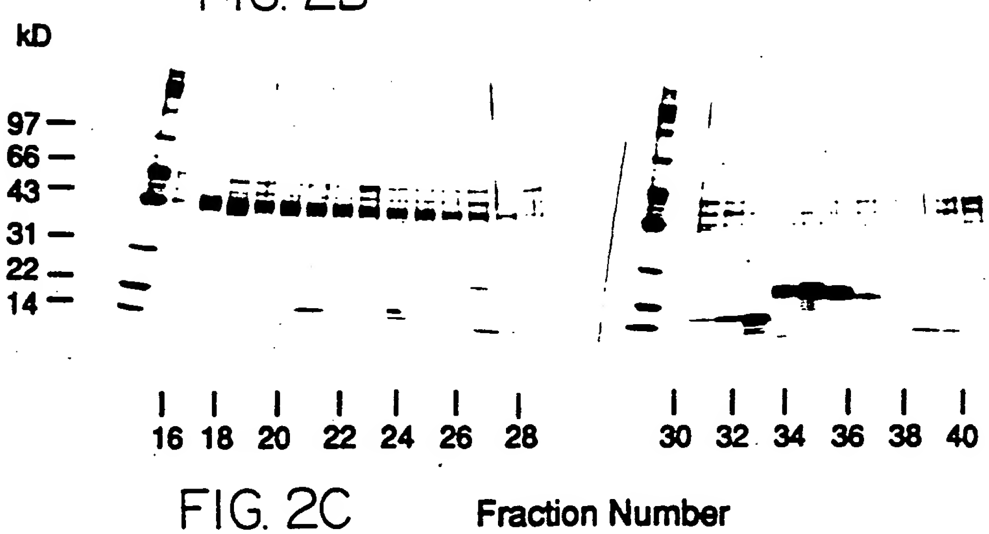

FIG. 2C

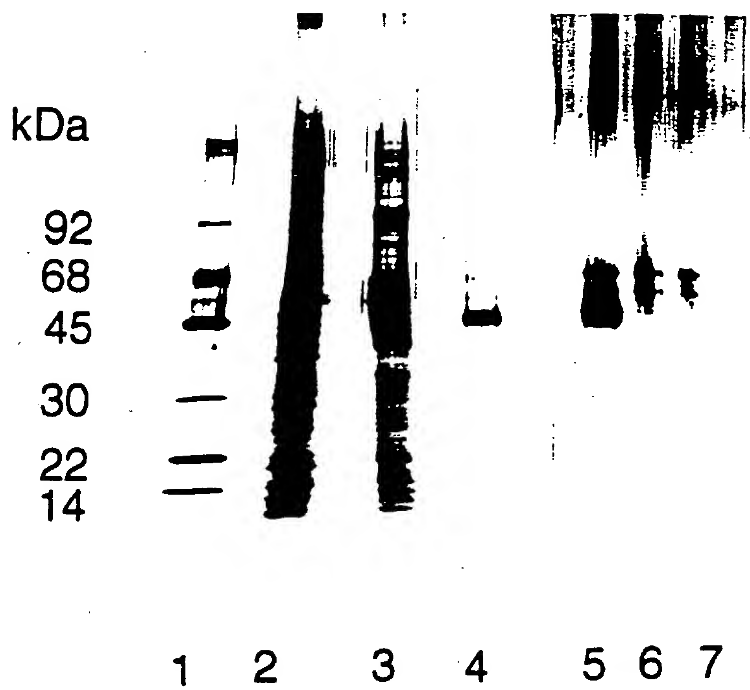

FIG. 3

```
GG  GCG CGA GCG CCT CAG CGC GGC CGC TCG CTC TCC CCC 38
    Ala Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro
     1               5                  10

TCG AGG GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT 77
Ser Arg Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu
         15                  20                  25

GGA CCA AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG 116
Gly Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu
                 30                  35

CGC TCC GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC 155
Arg Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly
     40                  45                  50

AGA GGC AAA GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC 194
Arg Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser
             55                  60

GGC AAG AAG CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA 233
Gly Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro
 65                  70                  75

GCC TTG CCT CCC CGA TTG AAA GAG ATG AAA AGC CAG GAA 272
Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
         80                  85                  90

TCG GCT GCA GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC 311
Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr
                 95                 100

AGT TCT GAA TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG 350
Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys
    105                 110                 115

AAT GGG AAT GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT 389
Asn Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn
            120                 125

ATC AAG ATA CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC 428
Ile Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg
130                 135                 140

ATT AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG 467
Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met
        145                 150                 155

TGC AAA GTG ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT 506
Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser
                160                 165
```

FIG. 4A

```
GCC AAT ATC ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT 545
Ala Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr
    170                 175                 180

GGT ATG CCA GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA 584
Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser
            185                 190

GAG TCT CCC ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA 623
Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala
195                 200                 205

AAT ACT TCT TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA 662
Asn Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr
        210                 215                 220

AGC CAT CTT GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC 701
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe
                225                 230

TGT GTG AAT GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT 740
Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu
    235                 240                 245

TCA AAC CCC TCG AGA TAC TTG TGC AAG TGC CAA CCT GGA 779
Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Gln Pro Gly
            250                 255

TTC ACT GGA GCA AGA TGT ACT GAG AAT GTG CCC ATG AAA 818
Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro Met Lys
260                 265                 270

GTC CAA AAC CAA GAA AAG GCG GAG GAG CTG TAC CAG AAG 857
Val Gln Asn Gln Glu Lys Ala Glu Glu Leu Tyr Gln Lys
        275                 280                 285

AGA GTG CTG ACC ATA ACC GGC ATC TGC ATC GCC CTC CTT 896
Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu
                290                 295

GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC TGC AAA ACC 935
Val Val Gly Ile Met Cys Val Val Ala Tyr Cys Lys Thr
    300                 305                 310

AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT CTT CGG CAG 974
Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln
            315                 320

AGC CTT CGG TCT GAA CGA AAC AAT ATG ATG AAC ATT GCC 1013
Ser Leu Arg Ser Glu Arg Asn Asn Met Met Asn Ile Ala
325                 330                 335
```

FIG. 4B

```
AAT GGG CCT CAC CAT CCT AAC CCA CCC CCC GAG AAT GTC 1052
Asn Gly Pro His His Pro Asn Pro Pro Pro Glu Asn Val
        340                 345                 350

CAG CTG GTG AAT CAA TAC GTA TCT AAA AAC GTC ATC TCC 1091
Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser
                355                 360

AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG ACA TCC TTT 1130
Ser Glu His Ile Val Glu Arg Glu Ala Glu Thr Ser Phe
    365                 370                 375

TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT CAC TCC ACT 1169
Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
            380                 385

ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG AGC AAC GGA 1208
Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly
390                 395                 400

CAC ACT GAA AGC ATC CTT TCC GAA AGC CAC TCT GTA ATC 1247
His Thr Glu Ser Ile Leu Ser Glu Ser His Ser Val Ile
        405                 410                 415

GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC AGC AGC CCA 1286
Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser Pro
                420                 425

ACT GGG GGC CCA AGA GGA CGT CTT AAT GGC ACA GGA GGC 1325
Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Thr Gly Gly
    430                 435                 440

CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA GAA 1364
Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu
            445                 450

ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT AGT GAA AGG 1403
Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser Glu Arg
455                 460                 465

TAT GTG TCA GCC ATG ACC ACC CCG GCT CGT ATG TCA CCT 1442
Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro
        470                 475                 480

GTA GAT TTC CAC ACG CCA AGC TCC CCC AAA TCG CCC CCT 1481
Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro
                485                 490

TCG GAA ATG TCT CCA CCC GTG TCC AGC ATG ACG GTG TCC 1520
Ser Glu Met Ser Pro Pro Val Ser Ser Met Thr Val Ser
    495                 500                 505
```

FIG. 4C

```
ATG CCT TCC ATG GCG GTC AGC CCC TTC ATG GAA GAA GAG 1559
Met Pro Ser Met Ala Val Ser Pro Phe Met Glu Glu Glu
            510                 515

AGA CCT CTA CTT CTC GTG ACA CCA CCA AGG CTG CGG GAG 1598
Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu Arg Glu
520                 525                 530

AAG AAG TTT GAC CAT CAC CCT CAG CAG TTC AGC TCC TTC 1637
Lys Lys Phe Asp His His Pro Gln Gln Phe Ser Ser Phe
        535                 540                 545

CAC CAC AAC CCC GCG CAT GAC AGT AAC AGC CTC CCT GCT 1676
His His Asn Pro Ala His Asp Ser Asn Ser Leu Pro Ala
                550                 555

AGC CCC TTG AGG ATA GTG GAG GAT GAG GAG TAT GAA ACG 1715
Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr
    560                 565                 570

ACC CAA GAG TAC GAG CCA GCC CAA GAG CCT GTT AAG AAA 1754
Thr Gln Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys
            575                 580

CTC GCC AAT AGC CGG CGG GCC AAA AGA ACC AAG CCC AAT 1793
Leu Ala Asn Ser Arg Arg Ala Lys Arg Thr Lys Pro Asn
585                 590                 595

GGC CAC ATT GCT AAC AGA TTG GAA GTG GAC AGC AAC ACA 1832
Gly His Ile Ala Asn Arg Leu Glu Val Asp Ser Asn Thr
        600                 605                 610

AGC TCC CAG AGC AGT AAC TCA GAG AGT GAA ACA GAA GAT 1871
Ser Ser Gln Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp
                615                 620

GAA AGA GTA GGT GAA GAT ACG CCT TTC CTG GGC ATA CAG 1910
Glu Arg Val Gly Glu Asp Thr Pro Phe Leu Gly Ile Gln
    625                 630                 635

AAC CCC CTG GCA GCC AGT CTT GAG GCA ACA CCT GCC TTC 1949
Asn Pro Leu Ala Ala Ser Leu Glu Ala Thr Pro Ala Phe
            640                 645

CGC CTG GCT GAC AGC AGG ACT AAC CCA GCA GGC CGC TTC 1988
Arg Leu Ala Asp Ser Arg Thr Asn Pro Ala Gly Arg Phe
650                 655                 660

TCG ACA CAG GAA GAA ATC CAG G 2010
Ser Thr Gln Glu Glu Ile Gln
        665             669
```

FIG. 4D

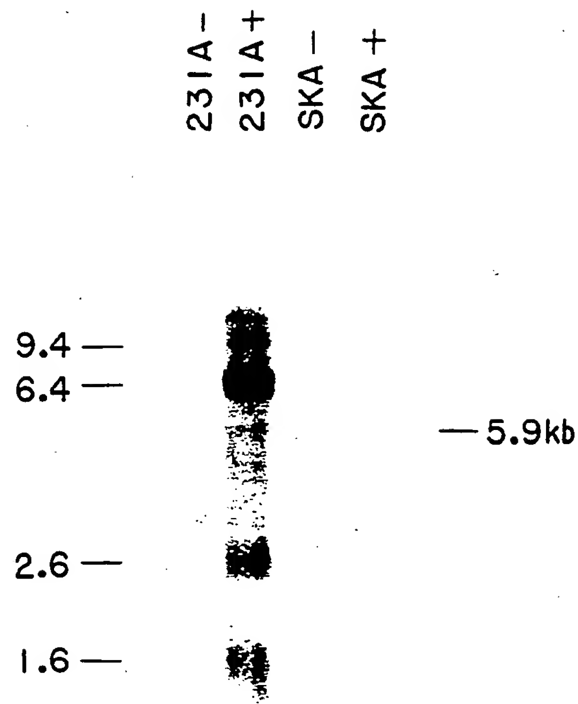

FIG. 5

9/32-

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             221       230       240       250       260
HRG2-alpha   S H L V K C A E K E K T F C V N G G E C F M V K D L S N P S R Y L C K C Q P G F T G A R C T E N
EGF          N S D S E C P L S H D G Y C L H D G V C M Y I E A L - - - D K Y A C N C V V G Y I G E R C Q Y R
TGF-alpha        N D C P D S H T Q F C F H - G T C R F L V Q E - - - D K P A C V C H S G Y V G A R C E H A
Amphiregulin K K K N P C N A E F Q N F C I H - G E C K Y I E H L - - - E A V T C K C Q Q E Y F G E R C G E K
Schwannoma   K K K N P C A A K F Q N F C I H - G E C R Y I E N L - - - E V V T C H C H Q D Y F G E R C G E K
HB-EGF       K K R D P C L R K Y K D F C I H - G E C K Y V K E L - - - R A P S C I C H P G Y H G E R C H G L

             270       280       290       300       310
HRG2-alpha   V P M K V Q N Q E K A E E L Y Q K R V L T I T G I C I A L L V V G I M C V V A Y C K T K K Q R .
EGF          D L K W W E L R H A G H G Q Q Q - K V I V V A V C V V V L V M L L L L S L W G A H Y Y R T Q K
TGF-alpha    D L L A V V A A S Q K - - - - - - K Q A I T A L V V V S I V A L A V L I I T C V L I H C C Q V
Amphiregulin S M K T H S M I D S S L S - - - - K I A L A A I A A F M S A V I L T A V A V I T V Q L R R Q Y
Schwannoma   T M K T Q K K D D S D L S - - - - K I A L A A I I V F V S A V S V A A I G I I T A V L L R K R
HB-EGF       S L P V E N R L Y T Y D - - - - - H T T I L A V V A V V L S S V C L L V I V G L L M F R Y H R
                                                 TRANSMEMBRANE REGION
```

FIG. 6

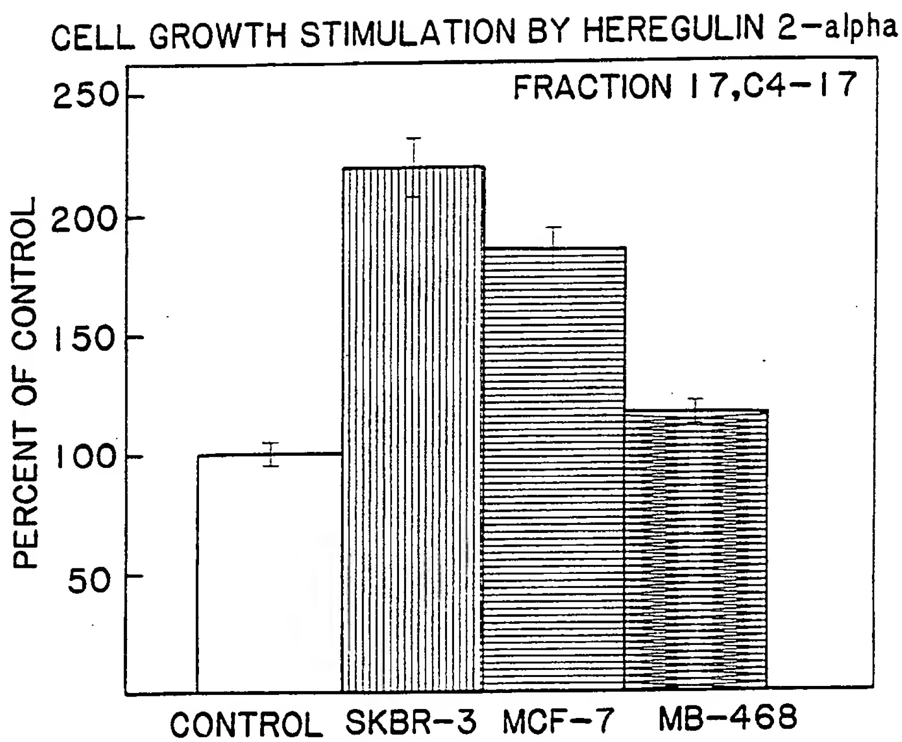
CELL GROWTH STIMULATION BY HEREGULIN 2-alpha
FRACTION 17,C4-17
PERCENT OF CONTROL
250
200
150
100
50
CONTROL
SKBR-3
MCF-7
MB-468

FIG. 7

```
GG  GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT GGA 38
    Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly
     1               5                  10

CCA AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG CGC 77
Pro Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu Arg
         15                  20                  25

TCC GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC AGA 116
Ser Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly Arg
                 30                  35

GGC AAA GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC 155
Gly Lys Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly
     40                  45                  50

AAG AAG CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA GCC 194
Lys Lys Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala
             55                  60

TTG CCT CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG 233
Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser
 65                  70                  75

GCT GCA GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT 272
Ala Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser
         80                  85                  90

TCT GAA TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG AAT 311
Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
                 95                 100

GGG AAT GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT ATC 350
Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile
    105                 110                 115

AAG ATA CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC ATT 389
Lys Ile Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile
            120                 125

AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC 428
Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
130                 135                 140

AAA GTG ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC 467
Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala
        145                 150                 155

AAT ATC ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT GGT 506
Asn Ile Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly
                160                 165
```

FIG. 8A

```
ATG CCA GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA GAG 545
Met Pro Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu
    170                 175                 180

TCT CCC ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA AAT 584
Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn
            185                 190

ACT TCT TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA AGC 623
Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser
195                 200                 205

CAT CTT GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT 662
His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys
        210                 215                 220

GTG AAT GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA 701
Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
                225                 230

AAC CCC TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT 740
Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe
    235                 240                 245

ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC 779
Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
            250                 255

TAC AAG CAT CTT GGG ATT GAA TTT ATG GAG GCG GAG GAG 818
Tyr Lys His Leu Gly Ile Glu Phe Met Glu Ala Glu Glu
260                 265                 270

CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC TGC 857
Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys
        275                 280                 285

ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC 896
Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala
                290                 295

TAC TGC AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC 935
Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp
    300                 305                 310

CGT CTT CGG CAG AGC CTT CGG TCT GAA CGA AAC AAT ATG 974
Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met
            315                 320

ATG AAC ATT GCC AAT GGG CCT CAC CAT CCT AAC CCA CCC 1013
Met Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro
325                 330                 335
```

FIG. 8B

SUBSTITUTE SHEET

```
CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA TCT AAA 1052
Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys
        340                 345                 350

AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA 1091
Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala
                355                 360

GAG ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC 1130
Glu Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala
    365                 370                 375

CAT CAC TCC ACT ACT GTC ACC CAG ACT CCT AGC CAC AGC 1169
His His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser
            380                 385

TGG AGC AAC GGA CAC ACT GAA AGC ATC CTT TCC GAA AGC 1208
Trp Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser
390                 395                 400

CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG 1247
His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg
        405                 410                 415

CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT 1286
His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
                420                 425

GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG 1325
Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg
    430                 435                 440

CAT GCC AGA GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT 1364
His Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro
            445                 450

CAT AGT GAA AGG TAT GTG TCA GCC ATG ACC ACC CCG GCT 1403
His Ser Glu Arg Tyr Val Ser Ala Met Thr Thr Pro Ala
455                 460                 465

CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA AGC TCC CCC 1442
Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro
        470                 475                 480

AAA TCG CCC CCT TCG GAA ATG TCT CCA CCC GTG TCC AGC 1481
Lys Ser Pro Pro Ser Glu Met Ser Pro Pro Val Ser Ser
                485                 490

ATG ACG GTG TCC ATG CCT TCC ATG GCG GTC AGC CCC TTC 1520
Met Thr Val Ser Met Pro Ser Met Ala Val Ser Pro Phe
    495                 500                 505
```


FIG. 8C

```
ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG ACA CCA CCA 1559
Met Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro
            510                 515

AGG CTG CGG GAG AAG AAG TTT GAC CAT CAC CCT CAG CAG 1598
Arg Leu Arg Glu Lys Lys Phe Asp His His Pro Gln Gln
520                 525                 530

TTC AGC TCC TTC CAC CAC AAC CCC GCG CAT GAC AGT AAC 1637
Phe Ser Ser Phe His His Asn Pro Ala His Asp Ser Asn
        535                     540                 545

AGC CTC CCT GCT AGC CCC TTG AGG ATA GTG GAG GAT GAG 1676
Ser Leu Pro Ala Ser Pro Leu Arg Ile Val Glu Asp Glu
                550                     555

GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA GCC CAA GAG 1715
Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln Glu
    560                 565                 570

CCT GTT AAG AAA CTC GCC AAT AGC CGG CGG GCC AAA AGA 1754
Pro Val Lys Lys Leu Ala Asn Ser Arg Arg Ala Lys Arg
            575                 580

ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA TTG GAA GTG 1793
Thr Lys Pro Asn Gly His Ile Ala Asn Arg Leu Glu Val
585                 590                 595

GAC AGC AAC ACA AGC TCC CAG AGC AGT AAC TCA GAG AGT 1832
Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn Ser Glu Ser
        600                     605                 610

GAA ACA GAA GAT GAA AGA GTA GGT GAA GAT ACG CCT TTC 1871
Glu Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe
                615                     620

CTG GGC ATA CAG AAC CCC CTG GCA GCC AGT CTT GAG GCA 1910
Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser Leu Glu Ala
    625                 630                 635

ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG ACT AAC CCA 1949
Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg Thr Asn Pro
            640                     645

GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC CAG GCC AGG 1988
Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile Gln Ala Arg
650                 655                 660

CTG TCT AGT GTA ATT GCT AAC CAA GAC CCT ATT GCT GTA TA 2029
Leu Ser Ser Val Ile Ala Asn Gln Asp Pro Ile Ala Val
        665                     670                 675
```


FIG. 8D

```
HRGa   1  A R A P Q R G R S L S P S R D K L F P N P I R A L G P N S P A P R A V R V E R S V S G E M S E R K E
HRGb   1  - - - - - - - - - - - - - - D K L F P N P I R A L G P N S P A P R A V R V E R S V S G E M S E R K E

HRGa  51  G R G K G K G K K K E R G S G K K P E S A A G S Q S P A L P P R L K E M K S Q E S A A G S K L V L R
HRGb  37  G R G K G K G K K K E R G S G K K P E S A A G S Q S P A L P P Q L K E M K S Q E S A A G S K L V L R

HRGa 101  C E T S S E Y S S L R F K W F K N G N E L N R K N K P Q N I K I Q K K P G K S E L R I N K A S L A D
HRGb  87  C E T S S E Y S S L R F K W F K N G N E L N R K N K P Q N I K I Q K K P G K S E L R I N K A S L A D

HRGa 151  S G E Y M C K V I S K L G N D S A S A N I T I V E S N E I I T G M P A S T E G A Y V S S E S P I R I
HRGb 137  S G E Y M C K V I S K L G N D S A S A N I T I V E S N E I I T G M P A S T E G A Y V S S E S P I R I

HRGa 201  S V S T E G A N T S S S T S T S T T G T S H L V K C A E K E K T F C V N G G E C F M V K D L S N P S
HRGb 187  S V S T E G A N T S S S T S T S T T G T S H L V K C A E K E K T F C V N G G E C F M V K D L S N P S

HRGa 251  R Y L C K C Q P G F T G A R C T E N V P M K V Q N Q - - - - - E K A E E L Y Q K R V L T I T G I C I
HRGb 237  R Y L C K C P N E F T G D R C Q N Y V M A S F Y K H L G I E F M E A E E L Y Q K R V L T I T G I C I

HRGa 296  A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G P H H P N P
HRGb 287  A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G P H H P N P

HRGa 346  P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T T V T Q T P
HRGb 337  P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T T V T Q T P

HRGa 396  S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T G G P R E C
HRGb 387  S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T G G P R E C

HRGa 446  N S F L R H A R E T P D S Y R D S P H S E R Y V S A M T T P A R M S P V D F H T P S S P K S P P S E
HRGb 437  N S F L R H A R E T P D S Y R D S P H S E R Y V S A M T T P A R M S P V D F H T P S S P K S P P S E

HRGa 496  M S P P V S S M T V S M P S M A V S P F M E E E R P L L L V T P P R L R E K K F D H H P Q Q F S S F
HRGb 487  M S P P V S S M T V S M P S M A V S P F M E E E R P L L L V T P P R L R E K K F D H H P Q Q F S S F

HRGa 546  H H N P A H D S N S L P A S P L R I V E D E E Y E T T Q E Y E P A Q E P V K K L A N S R R A K R T K
HRGb 537  H H N P A H D S N S L P A S P L R I V E D E E Y E T T Q E Y E P A Q E P V K K L A N S R R A K R T K

HRGa 596  P N G H I A N R L E V D S N T S S Q S S N S E S E T E D E R V G E D T P F L G I Q N P L A A S L E A
HRGb 587  P N G H I A N R L E V D S N T S S Q S S N S E S E T E D E R V G E D T P F L G I Q N P L A A S L E A

HRGa 646  T P A F R L A D S R T N P A G R F S T Q E E I Q
HRGb 637  T P A F R L A D S R T N P A G R F S T Q E E I Q A R L S S V I A N Q D P I A V O N L N K H I D S P V

HRGb 687  K L Y F I O O S I P P O I K Q F I L F O Q F C K O K T G K K L L O I K Y M Y V K M K K K K K
```

FIG. 9

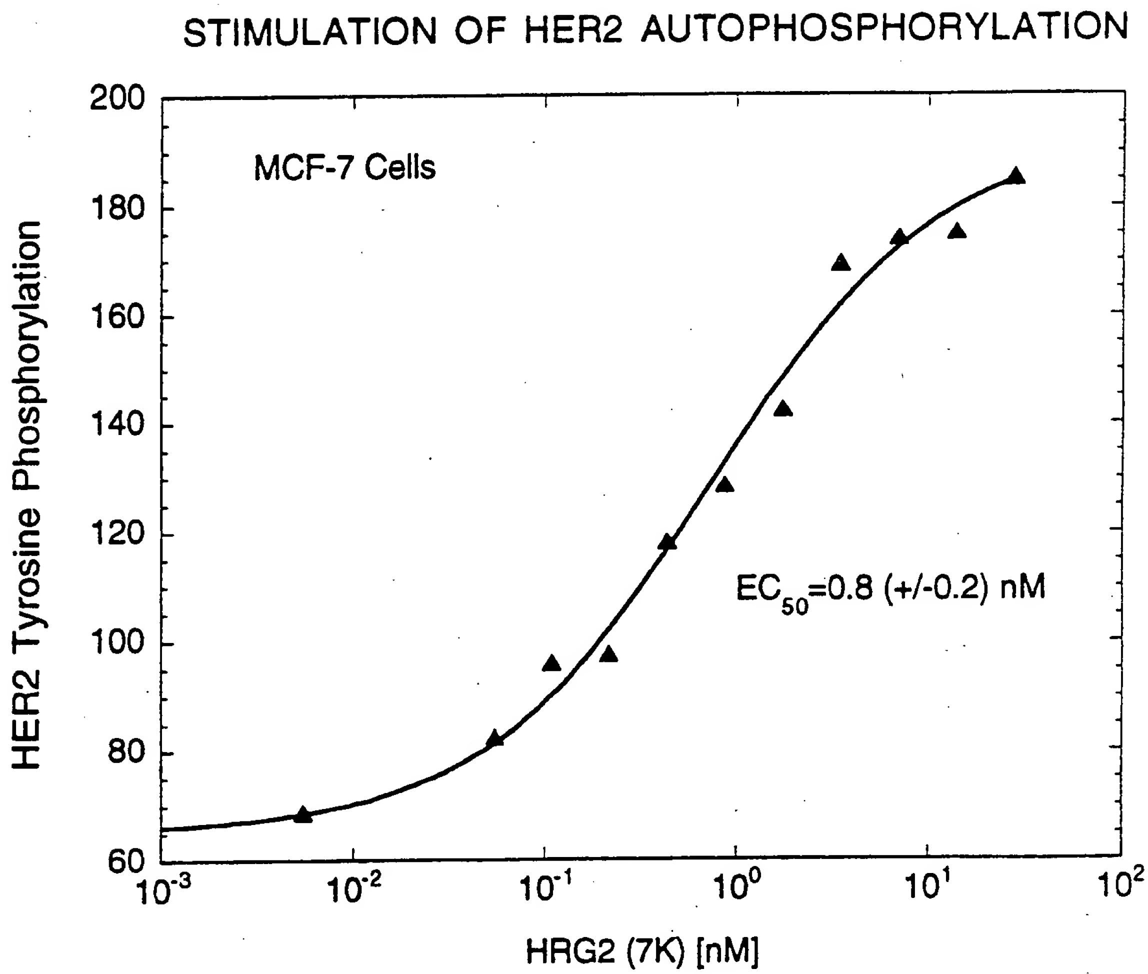
STIMULATION OF HER2 AUTOPHOSPHORYLATION
MCF-7 Cells
EC50=0.8 (+/-0.2) nM
HER2 Tyrosine Phosphorylation
200
180
160
140
120
100
80
60
10-3
10-2
10-1
100
101
102
HRG2 (7K) [nM]

FIG. 10

```
AA  AGA GCC GGC GAG GAG TTC CCC GAA ACT TGT TGG AAC 38
    Arg Ala Gly Glu Glu Phe Pro Glu Thr Cys Trp Asn
     1              5                   10

TCC GGG CTC GCG CGG AGG CCA GGA GCT GAG CGG CGG CGG 77
Ser Gly Leu Ala Arg Arg Pro Gly Ala Glu Arg Arg Arg
        15                  20                  25

CTG CCG GAC GAT GGG AGC GTG AGC AGG ACG GTG ATA ACC 116
Leu Pro Asp Asp Gly Ser Val Ser Arg Thr Val Ile Thr
                30                  35

TCT CCC CGA TCG GGT TGC GAG GGC GCC GGG CAG AGG CCA 155
Ser Pro Arg Ser Gly Cys Glu Gly Ala Gly Gln Arg Pro
    40                  45                  50

GGA CGC GAG CCG CCA GCG GTG GGA CCC ATC GAC GAC TTC 194
Gly Arg Glu Pro Pro Ala Val Gly Pro Ile Asp Asp Phe
            55                  60

CCG GGG CGA CAG GAG CAG CCC CGA GAG CCA GGG CGA GCG 233
Pro Gly Arg Gln Glu Gln Pro Arg Glu Pro Gly Arg Ala
65                  70                  75

CCC GTT CCA GGT GGC CGG ACC GCC CGC CGC GTC CGC GCC 272
Pro Val Pro Gly Gly Arg Thr Ala Arg Arg Val Arg Ala
        80                  85                  90

GCG CTC CCT GCA GGC AAC GGG AGA CGC CCC CGC GCA GCG 311
Ala Leu Pro Ala Gly Asn Gly Arg Arg Pro Arg Ala Ala
                95                  100

CGA GCG CCT CAG CGC GGC CGC TCG CTC TCC CCC TCG AGG 350
Arg Ala Pro Gln Arg Gly Arg Ser Leu Ser Pro Ser Arg
    105                 110                 115

GAC AAA CTT TTC CCA AAC CCG ATC CGA GCC CTT GGA CCA 389
Asp Lys Leu Phe Pro Asn Pro Ile Arg Ala Leu Gly Pro
            120                 125

AAC TCG CCT GCG CCG AGA GCC GTC CGC GTA GAG CGC TCC 428
Asn Ser Pro Ala Pro Arg Ala Val Arg Val Glu Arg Ser
130                 135                 140

GTC TCC GGC GAG ATG TCC GAG CGC AAA GAA GGC AGA GGC 467
Val Ser Gly Glu Met Ser Glu Arg Lys Glu Gly Arg Gly
        145                 150                 155

AAA GGG AAG GGC AAG AAG AAG GAG CGA GG 496
Lys Gly Lys Gly Lys Lys Lys Glu Arg
                160                 164
```

SUBSTITUTE SHEET FIG. 11

```
GTGGCTGCGG GGCAATTGAA AAAGAGCCGG CGAGGAGTTC CCCGAAACTT 50

GTTGGAACTC CGGGCTCGCG CGGAGGCCAG GAGCTGAGCG GCGGCGGCTG 100

CCGGACGATG GGAGCGTGAG CAGGACGGTG ATAACCTCTC CCCGATCGGG 150

TTGCGAGGGC GCCGGGCAGA GGCCAGGACG CGAGCCGCCA GCGGCGGGAC 200

CCATCGACGA CTTCCCGGGG CGACAGGAGC AGCCCCGAGA GCCAGGGCGA 250

GCGCCCGTTC CAGGTGGCCG GACCGCCCGC CGCGTCCGCG CCGCGCTC[illegible]C 300

TGCAGGCAAC GGGAGACGCC CCCGCGCAGC GCGAGCGCCT CAGCGCGGCC 350

GCTCGCTCTC CCCATCGAGG GACAAACTTT TCCCAAACCC GATCCGAGCC 400

CTTGGACCAA ACTCGCCTGC GCCGAGAGCC GTCCGCGTAG AGCGCTCCGT 450

CTCCGGCGAG  ATG TCC GAG CGC AAA GAA GGC AGA GGC AAA 490
            Met Ser Glu Arg Lys Glu Gly Arg Gly Lys
             1               5                   10

GGG AAG GGC AAG AAG AAG GAG CGA GGC TCC GGC AAG AAG 529
Gly Lys Gly Lys Lys Lys Glu Arg Gly Ser Gly Lys Lys
                 15                  20

CCG GAG TCC GCG GCG GGC AGC CAG AGC CCA GCC TTG CCT 568
Pro Glu Ser Ala Ala Gly Ser Gln Ser Pro Ala Leu Pro
     25                  30                  35

CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG GCT GCA 607
Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala
             40                  45

GGT TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT TCT GAA 646
Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
 50                  55                  60

TAC TCC TCT CTC AGA TTC AAG TGG TTC AAG AAT GGG AAT 685
Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn Gly Asn
         65                  70                  75

GAA TTG AAT CGA AAA AAC AAA CCA CAA AAT ATC AAG ATA 724
Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile
                 80                  85
```

FIG. 12A

```
CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC ATT AAC AAA 763
Gln Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys
     90                  95                 100

GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC AAA GTG 802
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
            105                 110

ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC AAT ATC 841
Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile
115                 120                 125

ACC ATC GTG GAA TCA AAC GAG ATC ATC ACT GGT ATG CCA 880
Thr Ile Val Glu Ser Asn Glu Ile Ile Thr Gly Met Pro
        130                 135                 140

GCC TCA ACT GAA GGA GCA TAT GTG TCT TCA GAG TCT CCC 919
Ala Ser Thr Glu Gly Ala Tyr Val Ser Ser Glu Ser Pro
                145                 150

ATT AGA ATA TCA GTA TCC ACA GAA GGA GCA AAT ACT TCT 958
Ile Arg Ile Ser Val Ser Thr Glu Gly Ala Asn Thr Ser
    155                 160                 165

TCA TCT ACA TCT ACA TCC ACC ACT GGG ACA AGC CAT CTT 997
Ser Ser Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu
            170                 175

GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT 1036
Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
180                 185                 190

GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA AAC CCC 1075
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro
        195                 200                 205

TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT 1114
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly
                210                 215

GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAG 1153
Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys
    220                 225                 230

GCG GAG GAG CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC 1192
Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr
            235                 240

GGC ATC TGC ATC GCC CTC CTT GTG GTC GGC ATC ATG TGT 1231
Gly Ile Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys
245                 250                 255

GTG GTG GCC TAC TGC AAA ACC AAG AAA CAG CGG AAA AAG 1270
Val Val Ala Tyr Cys Lys Thr Lys Lys Gln Arg Lys Lys
    260                 265                 270
```

FIG. 12B

CTG CAT GAC CGT CTT CGG CAG AGC CTT CGG TCT GAA CGA 1309
Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg
275 280

AAC AAT ATG ATG AAC ATT GCC AAT GGG CCT CAC CAT CCT 1348
Asn Asn Met Met Asn Ile Ala Asn Gly Pro His His Pro
285 290 295

AAC CCA CCC CCC GAG AAT GTC CAG CTG GTG AAT CAA TAC 1387
Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr
300 305

GTA TCT AAA AAC GTC ATC TCC AGT GAG CAT ATT GTT GAG 1426
Val Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu
310 315 320

AGA GAA GCA GAG ACA TCC TTT TCC ACC AGT CAC TAT ACT 1465
Arg Glu Ala Glu Thr Ser Phe Ser Thr Ser His Tyr Thr
325 330 335

TCC ACA GCC CAT CAC TCC ACT ACT GTC ACC CAG ACT CCT 1504
Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
340 345

AGC CAC AGC TGG AGC AAC GGA CAC ACT GAA AGC ATC CTT 1543
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Leu
350 355 360

TCC GAA AGC CAC TCT GTA ATC GTG ATG TCA TCC GTA GAA 1582
Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
365 370

AAC AGT AGG CAC AGC AGC CCA ACT GGG GGC CCA AGA GGA 1621
Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly
375 380 385

CGT CTT AAT GGC ACA GGA GGC CCT CGT GAA TGT AAC AGC 1660
Arg Leu Asn Gly Thr Gly Gly Pro Arg Glu Cys Asn Ser
390 395 400

TTC CTC AGG CAT GCC AGA GAA ACC CCT GAT TCC TAC CGA 1699
Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr Arg
405 410

GAC TCT CCT CAT AGT GAA AGG TAT GTG TCA GCC ATG ACC 1738
Asp Ser Pro His Ser Glu Arg Tyr Val Ser Ala Met Thr
415 420 425

ACC CCG GCT CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA 1777
Thr Pro Ala Arg Met Ser Pro Val Asp Phe His Thr Pro
430 435

AGC TCC CCC AAA TCG CCC CCT TCG GAA ATG TCT CCA CCC 1816
Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro Pro
440 445 450

FIG. 12C

GTG TCC AGC ATG ACG GTG TCC AAG CCT TCC ATG GCG GTC 1855
Val Ser Ser Met Thr Val Ser Lys Pro Ser Met Ala Val
455 460 465

AGC CCC TTC ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG 1894
Ser Pro Phe Met Glu Glu Glu Arg Pro Leu Leu Leu Val
470 475

ACA CCA CCA AGG CTG CGG GAG AAG AAG TTT GAC CAT CAC 1933
Thr Pro Pro Arg Leu Arg Glu Lys Lys Phe Asp His His
480 485 490

CCT CAG CAG TTC AGC TCC TTC CAC CAC AAC CCC GCG CAT 1972
Pro Gln Gln Phe Ser Ser Phe His His Asn Pro Ala His
495 500

GAC AGT AAC AGC CTC CCT GCT AGC CCC TTG AGG ATA GTG 2011
Asp Ser Asn Ser Leu Pro Ala Ser Pro Leu Arg Ile Val
505 510 515

GAG GAT GAG GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA 2050
Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro
520 525 530

GCC CAA GAG CCT GTT AAG AAA CTC GCC AAT AGC CGG CGG 2089
Ala Gln Glu Pro Val Lys Lys Leu Ala Asn Ser Arg Arg
535 540

GCC AAA AGA ACC AAG CCC AAT GGC CAC ATT GCT AAC AGA 2128
Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala Asn Arg
545 550 555

TTG GAA GTG GAC AGC AAC ACA AGC TCC CAG AGC AGT AAC 2167
Leu Glu Val Asp Ser Asn Thr Ser Ser Gln Ser Ser Asn
560 565

TCA GAG AGT GAA ACA GAA GAT GAA AGA GTA GGT GAA GAT 2206
Ser Glu Ser Glu Thr Glu Asp Glu Arg Val Gly Glu Asp
570 575 580

ACG CCT TTC CTG GGC ATA CAG AAC CCC CTG GCA GCC AGT 2245
Thr Pro Phe Leu Gly Ile Gln Asn Pro Leu Ala Ala Ser
585 590 595

CTT GAG GCA ACA CCT GCC TTC CGC CTG GCT GAC AGC AGG 2284
Leu Glu Ala Thr Pro Ala Phe Arg Leu Ala Asp Ser Arg
600 605

ACT AAC CCA GCA GGC CGC TTC TCG ACA CAG GAA GAA ATC 2323
Thr Asn Pro Ala Gly Arg Phe Ser Thr Gln Glu Glu Ile
610 615 620

CAG GCC AGG CTG TCT AGT GTA ATT GCT AAC CAA GAC CCT 2362
Gln Ala Arg Leu Ser Ser Val Ile Ala Asn Gln Asp Pro
625 630

FIG. 12D

```
ATT GCT GTA TAAAACCTA AATAAACACA TAGATTCACC TGTAAAACTT 2410
Ile Ala Val
635     637

TATTTTATAT AATAAAGTAT TCCACCTTAA ATTAAACAAT TTATTTTATT 2460

TTAGCAGTTC TGCAAATAAA AAAAAAAAAA 2490
```

FIG. 12E

```
GCGCCTGCCT CCAACCTGCG GGCGGGAGGT GGGTGGCTGC GGGGCAATTG 50

AAAAAGAGCC GGCGAGGAGT TCCCCGAAAC TTGTTGGAAC TCCGGGCTCG 100

CGCGGAGGCC AGGAGCTGAG CGGCGGCGGC TGCCGGACGA TGGGAGCGTG 150

AGCAGGACGG TGATAACCTC TCCCCGATCG GGTTGCGAGG GCGCCGGGCA 200

GAGGCCAGGA CGCGAGCCGC CAGCGGCGGG ACCCATCGAC GACTTCCCGG 250

GGCGACAGGA GCAGCCCCGA GAGCCAGGGC GAGCGCCCGT TCCAGGTGGC 300

CGGACCGCCC GCCGCGTCCG CGCCGCGCTC CCTGCAGGCA ACGGGAGACG 350

CCCCCGCGCA GCGCGAGCGC CTCAGCGCGG CCGCTCGCTC TCCCCATCGA 400

GGGACAAACT TTTCCCAAAC CCGATCCGAG CCCTTGGACC AAACTCGCCT 450

GCGCCGAGAG CCGTCCGCGT AGAGCGCTCC GTCTCCGGCG AG   ATG 495
                                                 Met
                                                   1

TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 534
Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
             5                  10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 573
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 612
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
        30                  35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 651
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 690
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
    55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 729
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
             70                  75
```

FIG. 13A

```
AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 768
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
80                  85                  90

GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 807
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
        95                  100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 846
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                    110             115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 885
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 924
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
            135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 963
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1002
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1041
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1080
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1119
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1158
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC 1197
Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro
        225                 230                 235

TTT CTG TCT CTG CCT GAA TAGGA GCATGCTCAG TTGGTGCTGC 1240
Phe Leu Ser Leu Pro Glu
                240 241

TTTCTTGTTG CTGCATCTCC CCTCAGATTC CACCTAGAGC TAGATGTGTC 1290
```

FIG. 13B

TTACCAGATC TAATATTGAC TGCCTCTGCC TGTCGCATGA GAACATTAAC 1340

AAAAGCAATT GTATTACTTC CTCTGTTCGC GACTAGTTGG CTCTGAGATA 1390

CTAATAGGTG TGTGAGGCTC CGGATGTTTC TGGAATTGAT ATTGAATGAT 1440

GTGATACAAA TTGATAGTCA ATATCAAGCA GTGAAATATG ATAATAAAGG 1490

CATTTCAAAG TCTCACTTTT ATTGATAAAA TAAAAATCAT TCTACTGAAC 1540

AGTCCATCTT CTTTATACAA TGACCACATC CTGAAAAGGG TGTTGCTAAG 1590

CTGTAACCGA TATGCACTTG AAATGATGGT AAGTTAATTT TGATTCAGAA 1640

TGTGTTATTT GTCACAAATA AACATAATAA AAGGAGTTCA GATGTTTTTC 1690

TTCATTAACC AAAAAAAAAA AAAAA 1715

FIG. 13C

```
GAGGCGCCTG CCTCCAACCT GCGGGCGGGA GGTGGGTGGC TGCGGGGCAA 50

TTGAAAAAGA GCCGGCGAGG AGTTCCCCGA AACTTGTTGG AACTCCGGGC 100

TCGCGCGGAG GCCAGGAGCT GAGCGGCGGC GGCTGCCGGA CGATGGGAGC 150

GTGAGCAGGA CGGTGATAAC CTCTCCCCGA TCGGGTTGCG AGGGCGCCGG 200

GCAGAGGCCA GGACGCGAGC CGCCAGCGGC GGGACCCATC GACGACTTCC 250

CGGGGCGACA GGAGCAGCCC CGAGAGCCAG GGCGAGCGCC CGTTCCAGGT 300

GGCCGGACCG CCCGCCGCGT CCGCGCCGCG CTCCCTGCAG GCAACGGGAG 350

ACGCCCCCGC GCAGCGCGAG CGCCTCAGCG CGGCCGCTCG CTCTCCCCAT 400

CGAGGGACAA ACTTTTCCCA AACCCGATCC GAGCCCTTGG ACCAAACTCG 450

CCTGCGCCGA GAGCCGTCCG CGTAGAGCGC TCCGTCTCCG GCGAG    AT 497
                                                   Met
                                                     1

G TCC GAG CGC AAA GAA GGC AGA GGC AAA GGG AAG GGC AAG 537
  Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys
                  5                   10

AAG AAG GAG CGA GGC TCC GGC AAG AAG CCG GAG TCC GCG 576
Lys Lys Glu Arg Gly Ser Gly Lys Lys Pro Glu Ser Ala
 15                  20                  25

GCG GGC AGC CAG AGC CCA GCC TTG CCT CCC CAA TTG AAA 615
Ala Gly Ser Gln Ser Pro Ala Leu Pro Pro Gln Leu Lys
         30                  35                  40

GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT TCC AAA CTA 654
Glu Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu
                 45                  50

GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC 693
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
     55                  60                  65

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA 732
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg
             70                  75
```

FIG. 14A

```
AAA AAC AAA CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA 771
Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln Lys Lys Pro
 80                 85                  90

GGG AAG TCA GAA CTT CGC ATT AAC AAA GCA TCA CTG GCT 810
Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
         95                 100                 105

GAT TCT GGA GAG TAT ATG TGC AAA GTG ATC AGC AAA TTA 849
Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
                110                 115

GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG GAA 888
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu
    120                 125                 130

TCA AAC GAG ATC ATC ACT GGT ATG CCA GCC TCA ACT GAA 927
Ser Asn Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu
            135                 140

GGA GCA TAT GTG TCT TCA GAG TCT CCC ATT AGA ATA TCA 966
Gly Ala Tyr Val Ser Ser Glu Ser Pro Ile Arg Ile Ser
145                 150                 155

GTA TCC ACA GAA GGA GCA AAT ACT TCT TCA TCT ACA TCT 1005
Val Ser Thr Glu Gly Ala Asn Thr Ser Ser Ser Thr Ser
        160                 165                 170

ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA AAA TGT GCG 1044
Thr Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala
                175                 180

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1083
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
    185                 190                 195

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG 1122
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
            200                 205

TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA 1161
Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln
210                 215                 220

AAC TAC GTA ATG GCC AGC TTC TAC AAG GCG GAG GAG CTG 1200
Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu
        225                 230                 235

TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC TGC ATC 1239
Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile
                240                 245

GCC CTC CTT GTG GTC GGC ATC ATG TGT GTG GTG GCC TAC 1278
Ala Leu Leu Val Val Gly Ile Met Cys Val Val Ala Tyr
    250                 255                 260
```

FIG. 14B

```
TGC AAA ACC AAG AAA CAG CGG AAA AAG CTG CAT GAC CGT 1317
Cys Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg
            265                 270

CTT CGG CAG AGC CTT CGG TCT GAA CGA AAC AAT ATG ATG 1356
Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn Asn Met Met
275                 280                 285

AAC ATT GCC AAT GGG CCT CAC CAT CCT AAC CCA CCC CCC 1395
Asn Ile Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
        290                 295                 300

GAG AAT GTC CAG CTG GTG AAT CAA TAC GTA TCT AAA AAC 1434
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn
                305                 310

GTC ATC TCC AGT GAG CAT ATT GTT GAG AGA GAA GCA GAG 1473
Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
    315                 320                 325

ACA TCC TTT TCC ACC AGT CAC TAT ACT TCC ACA GCC CAT 1512
Thr Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His
            330                 335

CAC TCC ACT ACT GTC ACC CAG ACT CCT AGC CAC AGC TGG 1551
His Ser Thr Thr Val Thr Gln Thr Pro Ser His Ser Trp
340                 345                 350

AGC AAC GGA CAC ACT GAA AGC ATC CTT TCC GAA AGC CAC 1590
Ser Asn Gly His Thr Glu Ser Ile Leu Ser Glu Ser His
        355                 360                 365

TCT GTA ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC 1629
Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His
                370                 375

AGC AGC CCA ACT GGG GGC CCA AGA GGA CGT CTT AAT GGC 1668
Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly
    380                 385                 390

ACA GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT 1707
Thr Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His
            395                 400

GCC AGA GAA ACC CCT GAT TCC TAC CGA GAC TCT CCT CAT 1746
Ala Arg Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His
405                 410                 415

AGT GAA AGG TAAAA CCGAAGGCAA AGCTACTGCA GAGGAGAAAC 1790
Ser Glu Arg
        420
```

FIG. 14C

SUBSTITUTE SHEET

```
TCAGTCAGAG AATCCCTGTG AGCACCTGCG GTCTCACCTC AGGAAATCTA 1840
CTCTAATCAG AATAAGGGGC GGCAGTTACC TGTTCTAGGA GTGCTCCTAG 1890
TTGATGAAGT CATCTCTTTG TTTGACGGAA CTTATTTCTT CTGAGCTTCT 1940
CTCGTCGTCC CAGTGACTGA CAGGCAACAG ACTCTTAAAG AGCTGGGATG 1990
CTTTGATGCG GAAGGTGCAG CACATGGAGT TTCCAGCTCT GGCCATGGGC 2040
TCAGACCCAC TCGGGGTCTC AGTGTCCTCA GTTGTAACAT TAGAGAGATG 2090
GCATCAATGC TTGATAAGGA CCCTTCTATA ATTCCAATTG CCAGTTATCC 2140
AAACTCTGAT TCGGTGGTCG AGCTGGCCTC GTGTTCTTAT CTGCTAACCC 2190
TGTCTTACCT TCCAGCCTCA GTTAAGTCAA ATCAAGGGCT ATGTCATTGC 2240
TGAATGTCAT GGGGGGCAAC TGCTTGCCCT CCACCCTATA GTATCTATTT 2290
TATGAAATTC CAAGAAGGGA TGAATAAATA AATCTCTTGG ATGCTGCGTC 2340
TGGCAGTCTT CACGGGTGGT TTTCAAAGCA GAAAAAAAAA AAAAAAAAAA 2390
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 2431
```

FIG. 14D

30/32

16	1	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPRLKEMKSQESAAG
11	1	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
76	1	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
84	1	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG
78	1	MSERKEGRGKGKGKKKERGSGKKPESAAGSQSPALPPQLKEMKSQESAAG

16	51	SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
11	51	SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
76	51	SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
84	51	SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN
78	51	SKLVLRCETSSEYSSLRFKWFKNGNELNRKNKPQNIKIQKKPGKSELRIN

16	101	KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
11	101	KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
76	101	KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
84	101	KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS
78	101	KASLADSGEYMCKVISKLGNDSASANITIVESNEIITGMPASTEGAYVSS

16	151	ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
11	151	ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
76	151	ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
84	151	ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK
78	151	ESPIRISVSTEGANTSSSTSTSTTGTSHLVKCAEKEKTFCVNGGECFMVK

FIG. 15A

```
16  201  D L S N P S R Y L C K C Q P G F T G A R C T E N V P M K V Q N Q - - - - - E K A E E L Y Q K R V L T
11  201  D L S N P S R Y L C K C P N E F T G D R C Q N Y V M A S F Y K H L G I E F M E A E E L Y Q K R V L T
76  201  D L S N P S R Y L C K C P N E F T G D R C Q N Y V M A S F Y K - - - - - - - - A E E L Y Q K R V L T
84  201  D L S N P S R Y L C K C P N E F T G D R C Q N Y V M A S F Y K - - - - - - - - A E E L Y Q K R V L T
78  201  D L S N P S R Y L C K C P N E F T G D R C Q N Y V M A S F Y S T S T P F L S L P E

16  246  I T G I C I A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G
11  251  I T G I C I A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G
76  243  I T G I C I A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G
84  243  I T G I C I A L L V V G I M C V V A Y C K T K K Q R K K L H D R L R Q S L R S E R N N M M N I A N G

16  296  P H H P N P P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T
11  301  P H H P N P P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T
76  293  P H H P N P P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T
84  293  P H H P N P P P E N V Q L V N Q Y V S K N V I S S E H I V E R E A E T S F S T S H Y T S T A H H S T

16  346  T V T Q T P S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T
11  351  T V T Q T P S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T
76  343  T V T Q T P S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T
84  343  T V T Q T P S H S W S N G H T E S I L S E S H S V I V M S S V E N S R H S S P T G G P R G R L N G T
```

FIG. 15B

16	396	GGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
11	401	GGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
76	393	GGPRECNSFLRHARETPDSYRDSPHSERYVSAMTTPARMSPVDFHTPSSP
84	393	GGPRECNSFLRHARETPDSYRDSPHSER----------------------
16	446	KSPPSEMSPPVSSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
11	451	KSPPSEMSPPVSSMTVSMPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
76	443	KSPPSEMSPPVSSMTVSKPSMAVSPFMEEERPLLLVTPPRLREKKFDHHP
16	496	QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
11	501	QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
76	493	QQFSSFHHNPAHDSNSLPASPLRIVEDEEYETTQEYEPAQEPVKKLANSR
16	546	RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLGIQNPL
11	551	RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLGIQNPL
76	543	RAKRTKPNGHIANRLEVDSNTSSQSSNSESETEDERVGEDTPFLGIQNPL
16	596	AASLEATPAFRLADSRTNPAGRFSTQEEIQ---------------
11	601	AASLEATPAFRLADSRTNPAGRFSTQEEIQARLSSVIANQDPIAV
76	593	AASLEATPAFRLADSRTNPAGRFSTQEEIQARLSSVIANQDPIAV

FIG. 15C

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/04295

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12P21/02; C12P21/08; C12N5/10
C12N1/21; A61K37/02; //(C12N1/21,C12R1:19)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
T	SCIENCE. vol. 256, 22 May 1992, LANCASTER, PA US pages 1205 - 1210 Holmes WE;Sliwkowski MX;Akita RW;Henzel WJ;Lee J;Park JW;Yansura D;Abadi N;Raab H;Lewis GD;et al 'Identification of heregulin, a specific activator of p185erbB2.' see the whole document -----	1-40

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 OCTOBER 1992	21. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.